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BACTERIAL STREAK DISEASE OF SORGHUMS[1]

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INTRODUCTION

Specimens of the disease here designated as bacterial streak disease were first seen by the writer on leaves of a feterita-milo hybrid (F. C. I.[3] 8926) sent by H. N. Vinall from Chillicothe, Tex., in 1921. The disease was found at Hays, Colby, and Manhattan, Kans., in 1924 on a large number of sorghum varieties, some of which were badly streaked with the disease from the lowest to the uppermost leaves. Specimens of the disease also have been sent in from Woodward, Okla., and Glendive and Huntley, Mont., and have been collected again recently at Chillicothe, Tex.

SYMPTOMS

The bacterial streak disease is characterized by dark reddish-brown streaks on the leaves of *Holcus sorghum* and *H. halepensis*. (Pl. 1; figs. 1, 2, and 3, B.)

The youngest lesions (fig. 2, A) are narrow water-soaked streaks 2 to 3 mm. broad and 2 to 15 cm. or more long. They show no red coloring and occur on plants from the seedling stage, with only two leaves, to those with heads nearing maturity.

Narrow red-brown margins or blotches of color soon appear in the water-soaked streaks (fig. 2, B) and in a few days the red color is continuous throughout the lesions. Older lesions are no longer translucent. (Fig. 2, C.) At intervals these red streaks broaden into elongated oval spots with tan centers and narrow red margins. (Pl. 1, B, and fig. 1.) These lesions, when numerous, coalesce to form long, irregular streaks and blotches covering a part or the whole width of the leaf blade and having more or less dead tissue with narrow dark margins between the reddish-brown streaks. Exudate is abundant (fig. 3, A), standing out on the young lesions as light-yellow beads which dry to thin white or cream-colored scales.

ISOLATIONS AND INOCULATIONS

Isolations were made both by washing the tissue through 12 sterile water blanks and by dipping it in 95 per cent alcohol and then for two minutes in 1:1,000 mercuric chloride and washing. These isolations have repeatedly given the same yellow colonies in either pure or mixed cultures.

[1] Received for publication Aug. 9, 1929; issued May, 1930.

[2] The writer wishes to express appreciation to A. F. Swanson, assistant agronomist of the Office of Cereal Crops and Diseases, for assistance in making the observations at Hays, Kans., and to J. F. Brewer, chief scientific illustrator of the Office of Mycology and Disease Survey, for making the colored plates and the photographs.

[3] F. C. I. indicates a serial number of the Office of Forage Crops and Diseases.

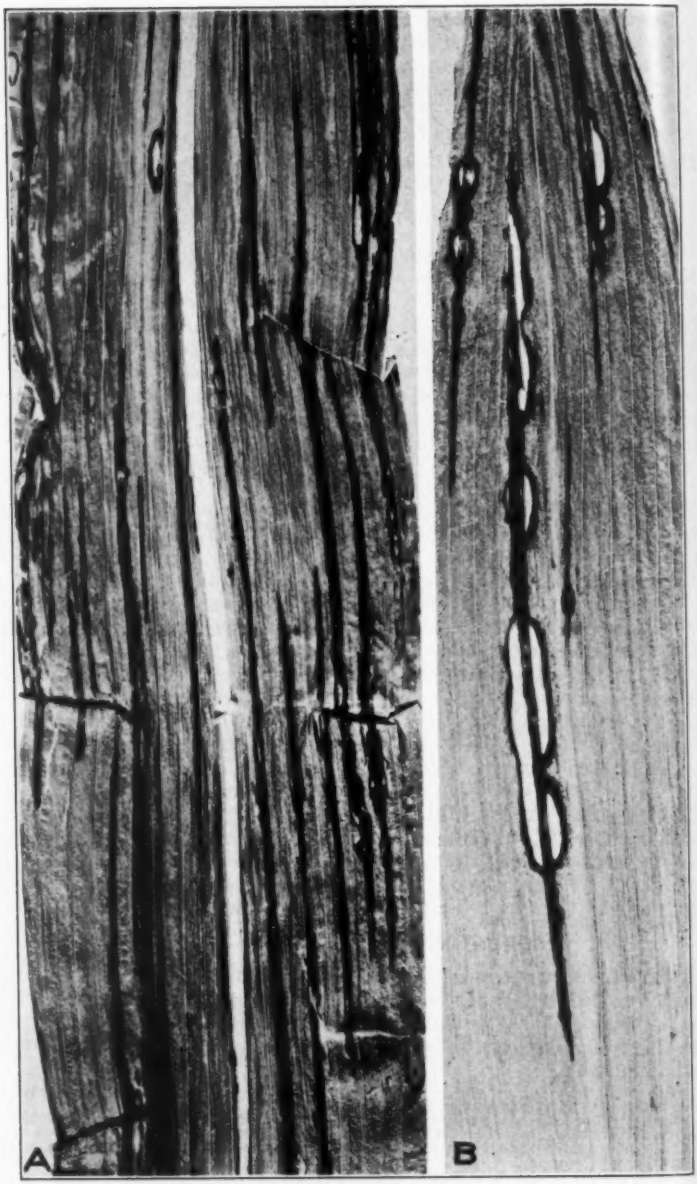


FIGURE 1.—Natural infections on Black Amber sorgo (A) and Wonder kafir (B). Collected at Hays, Kans., August 7, 1924. × 1

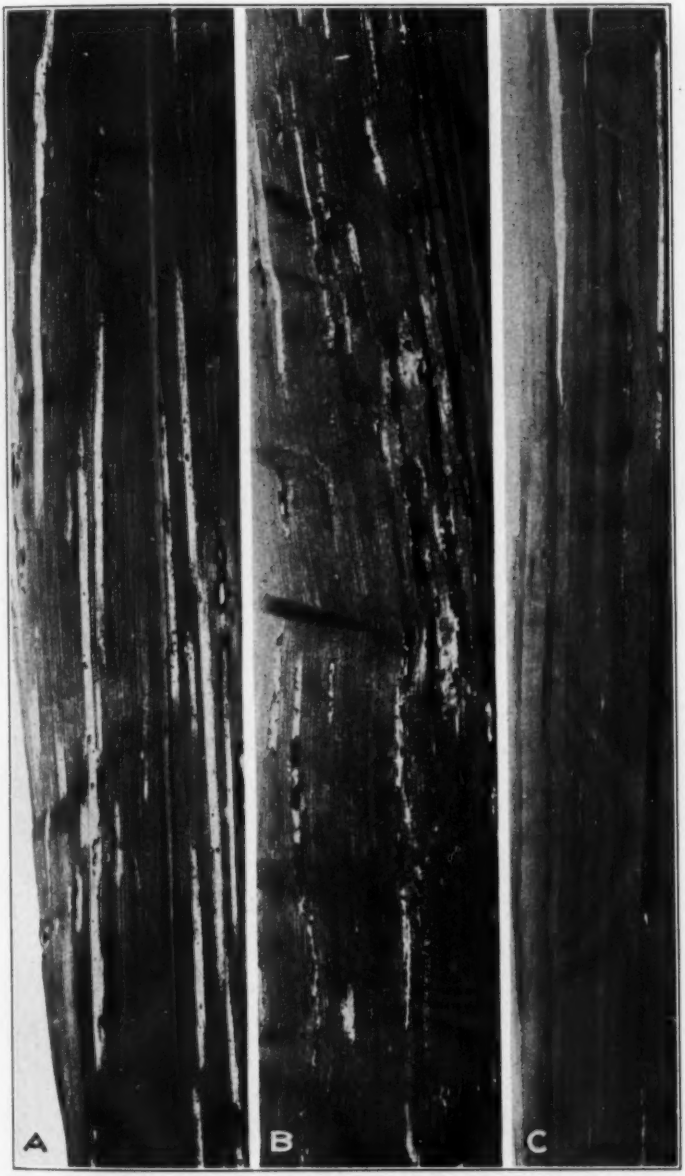


FIGURE 2.—Natural infections of different ages photographed by transmitted light to show different degrees of translucency. Collected at Hays, Kans., 1924. × 1. A, Young translucent lesions on Pink kafir × milo. B, Slightly older and only partly translucent lesions on Pink kafir × Dwarf milo. C, Older lesions, which are no longer translucent, on Farr's Dwarf hegari

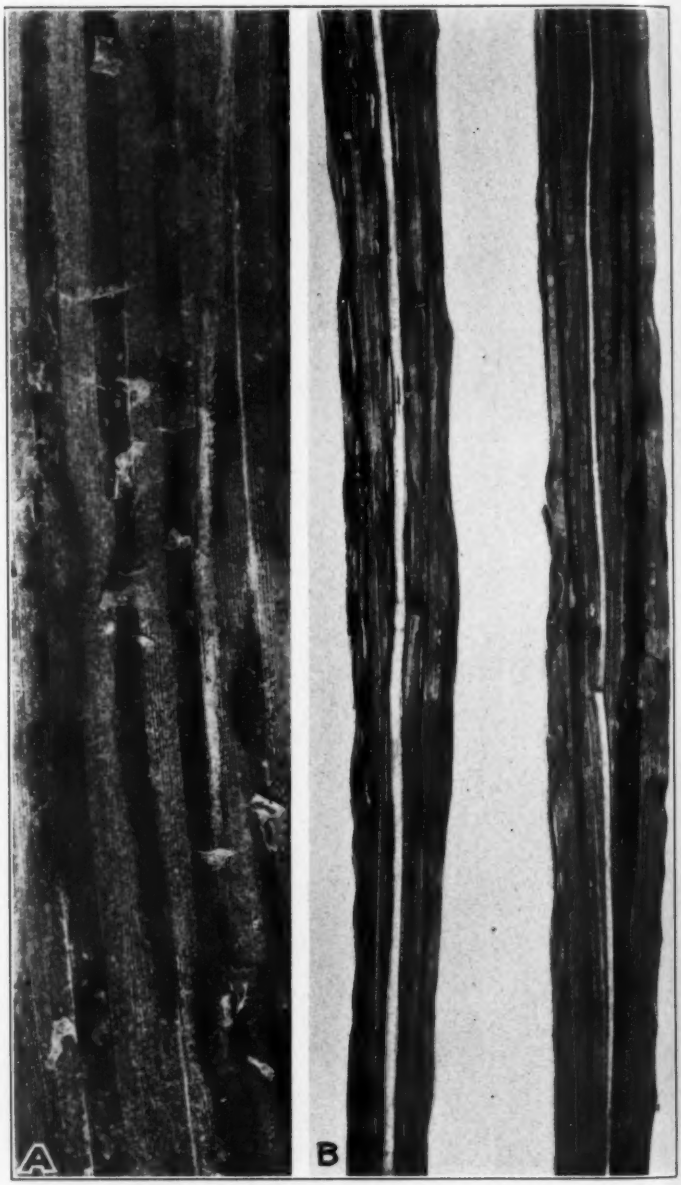
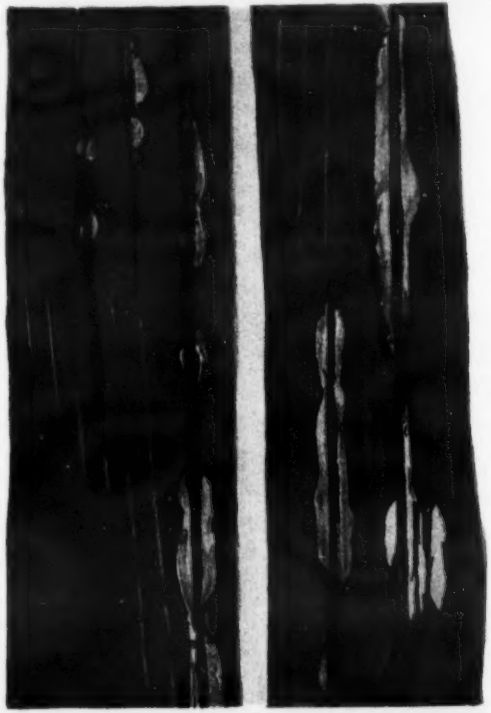


FIGURE 3.—A, Natural infection, with abundant exudate scales, on Pink kafir × milo. Collected at Hays, Kans., August 28, 1924. Photographed September 3, 1924. × 4. B, Natural infection on Johnson grass. Collected at Chillicothe, Tex., August, 1928. × 1

A



B

A, Young translucent lesions of bacterial streak on Pink kafir×Dwarf milo collected at Hays, Kans., 1924. × 1. B, Older lesions of bacterial streak on Wonder kafir collected at Hays, Kans., 1924, showing typical elongated oval spots along the streaks. ×1

Of the 60 isolations from typical lesions of this disease, 14 were made from the original material from Chillicothe, Tex., in 1921, 34 from material collected at Hays, Kans., in 1924, and the remainder from lesions collected at Chillicothe, Tex., at Woodward, Okla., at Glendive and Huntley, Mont., and at Rosslyn, Va. Recently the same organism has been isolated from the same type of lesion on Johnson grass collected at Chillicothe, Tex. Yellow colonies developed on all the plates from these lesions. Some of them appeared in one to two days, but the colonies of the parasite developed more slowly, becoming visible only on the third or fourth day and showing the light-yellow color only after they had reached a diameter of about 2 mm.

The persistent appearance of this slow-growing light-yellow organism in large numbers on the plates led to the assumption that it must be the cause of the disease, but proof of its pathogenicity was wanting for some time. Inoculations were made by spraying wounded and unwounded plants with water or broth suspensions from young agar cultures. The inoculated plants, together with the controls sprayed with sterile water, were held in damp chambers for several days after inoculation.

Fifteen inoculations from organisms isolated from the material collected in Texas in 1921, were made on feterita × milo, feterita, broomcorn, Dwarf kafir, an African sorghum collected by H. L. Shantz, Early Fortune proso (S. Dak. No. 98), and feterita × Red Amber. Not a single lesion developed on any of the plants. In the field pieces of infected leaf tissue with abundant exudate were tied to healthy leaves, which were pierced with a needle and covered with moist cotton, but this procedure always failed to produce infection.

During the fall and winter of 1924–25 about 40 inoculations were made with isolations from materials collected at Hays, Kans., in 1924. In only 6 of these inoculations were lesions produced on inoculated plants, and none of these artificial infections was more than 1 to 2 mm. broad and 6 to 20 mm. long. Exudate was present on two lesions and from sections under the microscope bacteria streamed in abundance. From three of these lesions typical cultures of the light-yellow organism were reisolated.

While isolations Nos. 72 and 80 in one experiment produced lesions up to 20 mm. long, these same cultures, tested twice afterwards under apparently similar conditions, failed to produce any infections.

In the fall of 1927 the same yellow organism was isolated from young lesions on Dwarf White milo collected at Chillicothe, Tex. This isolation, No. 89, was sprayed on plants of Dwarf White milo growing in temperature tanks at 24°, 32°, and 36° C. No lesions developed on plants growing at 32° or 36°, but on plants growing at 24° 10 small lesions developed. They were translucent, more or less red, 1 mm. wide, and up to 6 mm. in length. The typical yellow organism was reisolated. This same organism used for inoculation in December apparently produced no lesions, but four months later typical red lesions 2 to 10 cm. long were found on these plants. The plants were cut down before the organism was reisolated.

At the suggestion of A. G. Johnson, this same organism was used again for inoculation by spraying the plants after dark and covering them with inverted flower pots. Ten days after inoculation typical

lesions of the disease developed on several plants. The lesions were translucent, red along the margins or throughout, 3 to 4 mm. in diameter, and 10 to 15 cm. long. Pure cultures of the lemon-yellow organism were reisolated from three of these lesions. One of these reisolations again was used for spraying milo plants after dark. Also one set of plants was sprayed about noon and both sets were covered with earthen pots. (Fig. 4, A.) Both the plants inoculated after dark and those inoculated about noon developed a few small lesions 2 to 20 cm. long, from which the typical yellow colonies were reisolated.

The organism isolated from lesions on Johnson grass and sprayed on Dwarf White milo after dark has produced abundant typical infections (fig. 4, B), and the yellow organism has been reisolated and again sprayed on Dwarf Yellow milo. The pathogenicity of the organism thus has been established, but the conditions that limit infection have not been made clear. The only inoculation producing abundant infection was made after dark.

THE CAUSAL ORGANISM

MORPHOLOGY

Grown on beef-peptone agar and other nutrient media, the causal organism which is described technically as *Bacterium holcicola* n. sp. on page 972, is a short rod with rounded ends, arranged singly, in twos, or in threes. Grown on beef-infusion peptone agar and stained with carbol fuchsine, the rods vary in length from 1.05μ to 2.40μ and in diameter from 0.45μ to 0.90μ and average 0.65μ by 1.44μ. Grown on Thaxter's potato-dextrose agar and stained with carbol fuchsine, they vary in length from 1.3μ to 2.4μ and in diameter from 0.65μ to 1.1μ and average 0.84μ by 1.70μ. Capsules are present on beef-peptone agar cultures stained with Casares-Gil flagella stain and with Ribbert capsule stain. (Pl. 2, B.) Stained with the Casares-Gil flagella stain, the organism is shown to be motile on agar by 1 to 2 polar flagella. (Pl. 2, A, B.)

STAINING REACTIONS

Stained by the Hucker modification of the Gram stain, the causal organism is Gram-negative. Stained by the Ziehl-Neelsen method it is not acid-fast. It stains readily with carbol fuchsine and aniline gentian violet.

CULTURAL CHARACTERS

BEEF-INFUSION PEPTONE AGAR SLANT.—Growth is moderate, filiform, flat, glistening, smooth, translucent, light wax yellow,[4] and butyrous to slightly

[4] RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., illus. Washington, D. C., 1912.

EXPLANATORY LEGEND FOR PLATE 2

A and B.—Smears from 2-day agar slants of *Bacterium holcicola* (isolation No. 89). Stained with Casares Gil's stain, November 15, 1928. × 1,400.

C.—Seventeen-day colonies of *Bact. holcicola* (isolation No. 72) from Yellow milo, on beef-peptone agar, showing secondary growth in the original colonies. Photographed by transmitted light, February 17, 1925. × 4.

D.—Six-day colonies of *Bact. holcicola* (isolation No. 89) on beef-peptone agar. Photographed February 14, 1928, by transmitted light to show amorphous character. × 5.

E.—Six-day colonies of *Bact. holcicola* (isolation No. 89) on Thaxter's potato agar. Photographed February 14, 1928, by reflected light to show smooth surfaces and entire margins. × 5.

F.—Thirteen-day surface and embedded colonies of *Bact. holcicola* (isolation No. 95) on beef gelatin. Photographed February 13, 1928. × 2.

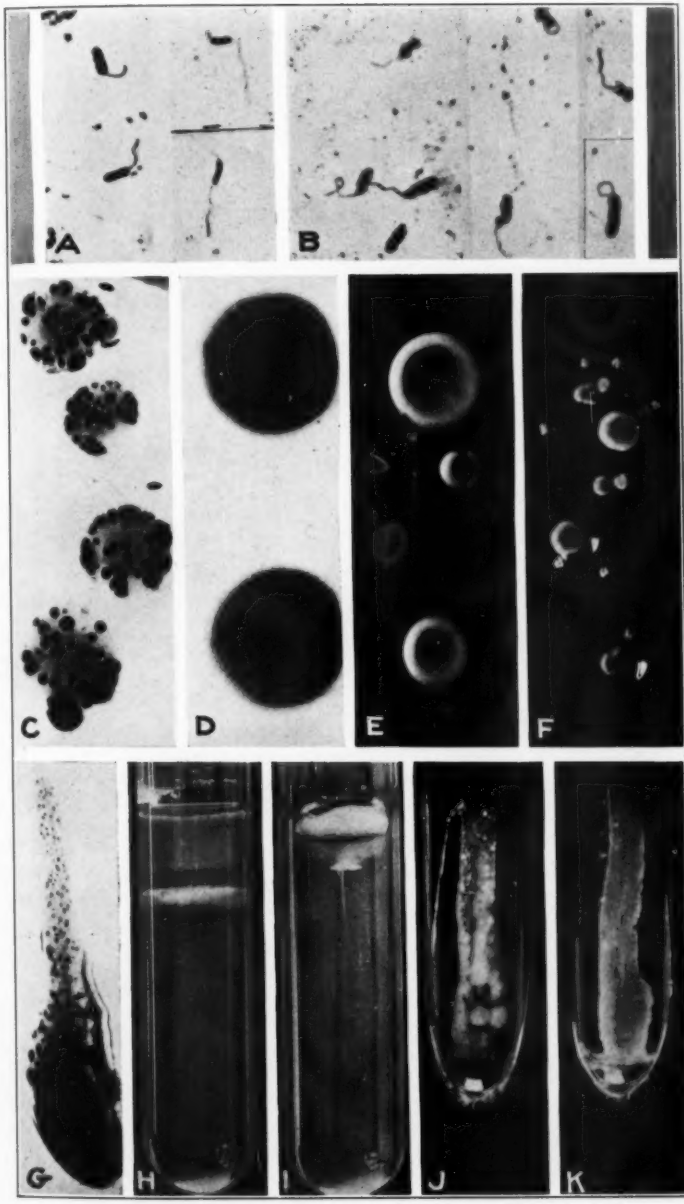
G.—Fourteen-day beef-peptone agar slant of *Bact. holcicola* (isolation No. 80). Photographed February 19, 1925, by transmitted light to show secondary colonies in original slant culture. × 2.

H.—Fourteen-day beef-gelatin stab of *Bact. holcicola*. Photographed February 14, 1928, to show stratiform liquefaction. × 1.

I.—Fourteen-day beef-gelatin stab of *Bact. holcicola* (isolation No. 89). Photographed February 14, 1928, to show napiform liquefaction. × 1.

J.—Twenty-day beef-peptone agar slant of *Bact. holcicola* (isolation No. 72), showing secondary colonies. Photographed March 21, 1925. × 1.

K.—Twenty-day beef-peptone agar slant of *Bact. andropogoni*. Photographed March 21, 1925. × 1.



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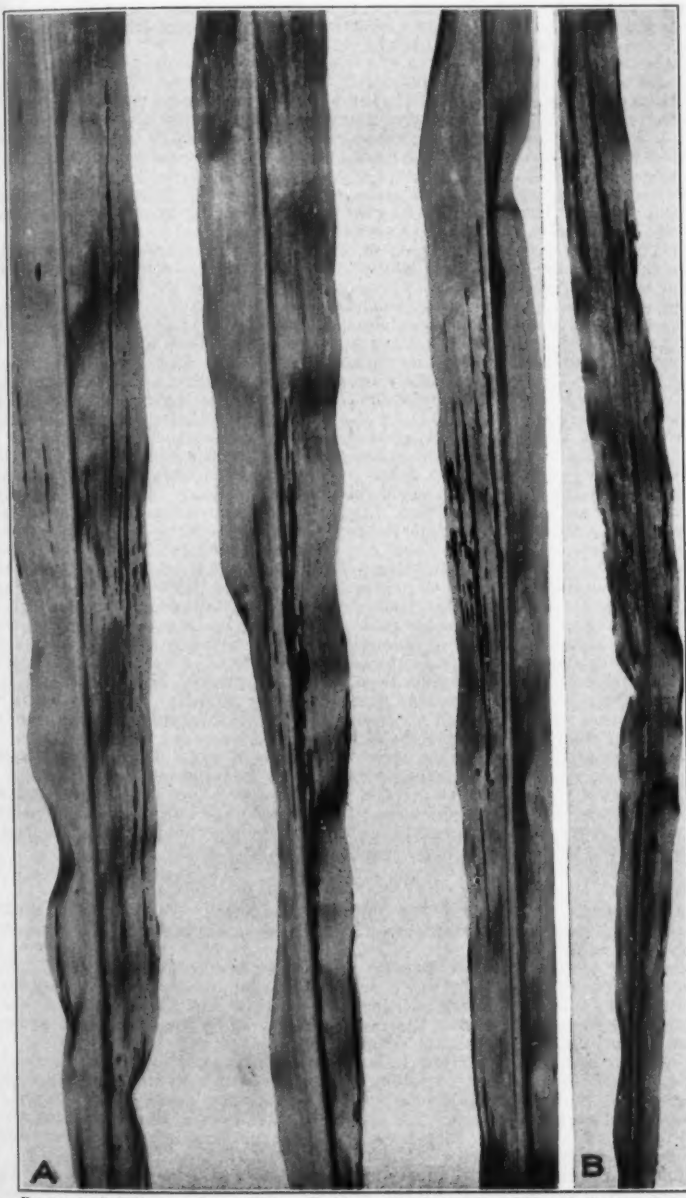

FIGURE 4.—Infections on Dwarf White milo from artificial spray inoculations in the greenhouse at Washington, D. C. A, Inoculated May 7, 1928, with culture No. 89, isolated from Early White milo (C. I. 480), collected at Woodward, Okla., September, 1926. Photographed June 5, 1928. × 1. B, Inoculated November 12 1928, with culture No. 95, isolated from Johnson grass, collected at Chillicothe, Tex. August, 1928. Photographed December 1, 1928. × 1

viscid in old cultures. The medium is unchanged. Secondary colonies begin to show in large numbers as tiny round colonies over the surface of the slant when the culture is about three weeks old. (Pl. 2, G, J.)

BEEF-INFUSION PEPTONE AGAR PLATES.—Colonies become visible in three to four days. They are round, with entire margins, umbonate, glistening, smooth, at first translucent, creamy white but in a few days becoming a light wax yellow and more opaque, butyrous, without internal markings except slight radiate lines. (Pl. 2, D, E.) After about two weeks, when the agar begins to dry, secondary colonies begin to develop along the margins and through the centers of the original colonies. (Pl. 2, C.)

THAXTER'S POTATO-DEXTROSE AGAR SLANTS.—Growth is abundant, raised, filiform to spreading, smooth, and glistening; a deep-cream color occurs along the slant and yellow in the condensation water at its base.

THAXTER'S POTATO-DEXTROSE AGAR PLATES.—Colonies are visible in two to three days. They are at first white to cream colored, umbonate, glistening, round with entire margins, amorphous to slightly radiate by transmitted light; after four to five days they become light yellow (pl. 2, E), and after two to three weeks secondary V-shaped colonies may begin to develop as on beef-infusion agar.

GELATIN STABS.—At room temperature growth is best at the top, and a colony of 2 to 3 mm. in diameter forms in two days. Liquefaction begins on the third day. The surface colony becomes slightly sunken and beneath it is produced a small pit of liquefaction into which the surface growth sinks. Liquefaction is napiform (pl. 2, I) for the first two weeks, after which it becomes stratiform. In three weeks the gelatin is about one-fourth liquefied.

BEEF GELATIN PLATES.—Small colonies appear on the third day. Colonies become bright yellow and more or less lobed around the margins, and about the tenth day they begin to sink into saucer-shaped depressions. (Pl. 2, F.) Heavily seeded plates are entirely liquefied. At the end of about three weeks scattered colonies are 4 to 5 mm. in diameter in depressions 8 to 10 mm. in diameter.

BEEF-INFUSION BOUILLON.—There is a trace of growth in 24 hours and light clouding in 48 hours with a scattered flocculence along one side of the tube from top to bottom. By the third day there forms at the surface a slight ring which is readily broken up into flocculent particles but which increases from day to day until it forms an irregular yellow ring and the clouding becomes moderate. A small quantity of sediment is formed which is viscid on agitation.

COHN'S SOLUTION.—Two cultures showed a trace of clouding in Cohn's solution, pH 5.2, in three to five days, but this gradually disappeared, and at the end of two weeks there was no evidence of any growth. Another test in a medium with a pH 5.3 showed no growth at all. The reaction of this medium is more acid than the minimum for this organism.

FERMI'S SOLUTION.—There is never more than a light clouding and after two months a viscid ring or pellicle strings down into the medium.

USCHINSKY'S SOLUTION.—At pH 6.4, there is a fine flocculent growth along the sides of the tube after eight days. This falls to the bottom and is more or less viscid. After about two months there is a very light clouding of the medium. In another test at pH 6.3 there was no growth at all.

PHYSIOLOGY

TEMPERATURE RELATIONS.—The optimum temperature for growth is about 28° to 30° C.; the maximum, 36° to 37°; and the minimum, 4°. The thermal death point is 51°.

RELATION TO REACTION OF MEDIUM.—Grown in beef-infusion peptone broth, the optimum reaction is pH 7.0 to 7.5, and the limits of growth are pH 5.5 and 9.0.

CHROMOGENESIS.—On nutrient agar the color of this organism is a wax yellow [5] and on potato-dextrose agar a deep cream along the streak and a wax yellow at the base. On potato the color is more of an empire yellow.

PRODUCTION OF INDOL.—Grown in Dunham's solution and tested with Ehrlich's test, the organism produced no indol, although a culture in which *Bacillus coli* was grown at the same time gave a positive reaction for indol. Grown in tryptophane broth and tested after 2 to 4 and 11 days by the Ehrlich-Böhme, the Goré modification of the Ehrlich-Böhme, and by the Gnezda technic, there was no reaction for indol.

PRODUCTION OF HYDROGEN SULPHIDE.—On lead-acetate agar stabs growth becomes dark brown at the surface and along the stab, indicating the formation of hydrogen sulphide.

[5] RIDGWAY, R. Op. cit.

RELATION TO OXYGEN.—In fermentation tubes containing sucrose, glucose, lactose, and glycerin there was growth only in the open arm. Shake cultures in beef-infusion peptone agar plus dextrose grew only at the surface. The organism is an aerobe.

MILK.—No curd is formed, and peptonization becomes evident about the sixth day and is completed in about 10 days. The reaction is the same in litmus milk. The color becomes a deeper shade of lavender or slightly more blue than the control and about the tenth day is reddish purple. By the sixteenth day the tubes are colorless. Old milk cultures become gelatinous.

NITRATE REDUCTION.—Three tests with nitrate broth gave no reaction for nitrites, although cultures of oat organisms in the same media gave definite evidence of reduction.

Two tests were made with isolations Nos. 89 and 95 on media recommended by the Society of American Bacteriologists.[6] The media were as follows: A, Nitrate-peptone broth; B, peptone broth without nitrate; C, a synthetic nitrate medium (KNO_3, 1 gm.; K_2HPO_4, 0.5 gm.; $CaCl_2$, 0.5 gm.; glucose, 10 gm.; distilled water, 1,000 c. c.); D, peptone broth with 2 parts per million potassium nitrite. No. 89 is an older culture and does not grow so vigorously as No. 95. This would account for slight differences in results with the two strains.

The organism grew well in A, B, and D, but there was only light clouding in the synthetic medium in both tests. In the first set the test for ammonia was made with strips of filter paper moistened with Nessler's solution and suspended over the cultures. A, B, and C were tested for ammonia. Uninoculated controls showed no reaction for ammonia. Cultures in the synthetic medium also showed no reaction for ammonia.

In 10 days both No. 89 and No. 95 showed slight browning of the filter paper in peptone broth. In nitrate broth there was no browning in No. 89 and decided browning of the lower edge in No. 95.

In 19 days in nitrate broth No. 89 showed a trace of browning on the edge of the filter paper and No. 95 turned the paper black for 5 to 6 mm.

In peptone broth the filter paper in both cultures was dark brown on the lower edge.

Tests for nitrites were made with sulphanilic acid and α-naphthylamine.

In the synthetic medium there was never any reaction for nitrites.

In D (peptone broth plus 2 parts per million nitrite) tests for nitrites were made on the first, second, fourth, seventh, thirteenth, and thirty-seventh days. Controls were always a deep-pink or rose color. Up to the thirteenth day the cultures were as deep pink as the controls, but on the thirty-seventh day No. 89 was light pink and No. 95 very light pink.

In the second test growth was the same as in the first. The Thomas test was used for ammonia and sulphanilic acid and α-naphthylamine for nitrites. The results of tests for ammonia in nitrate broth and in peptone broth are shown in Table 1.

TABLE 1.—*Results of Thomas tests for ammonia in nitrate broth and in peptone broth, uninoculated (control) and inoculated with Bacterium holcicola cultures Nos. 89 and 95, respectively*

Days after inoculation	Control or culture No.	Results of color tests for ammonia in—	
		Nitrate broth	Peptone broth
1	Control	Greenish	Trace green.
1	89	Slightly green	No color.
1	95	No color	Do.
3	Control	Greenish	Green.
3	89	do	Less green than control
3	95	Slightly bluer than control	Do.
7	Control	Slightly green	Slightly green.
7	89	No color	No color.
7	95	do	Do.
20	Control	Trace bluish throughout	No color.
20	89	Deep blue in lower third	Deep blue in lower fourth.
20	95	do	Do.

[6] SOCIETY OF AMERICAN BACTERIOLOGISTS. COMMITTEE ON BACTERIOLOGICAL TECHNIC. MANUAL OF METHODS FOR PURE CULTURE STUDY OF BACTERIA, FOR USE WITH THE DESCRIPTIVE CHART OF THE SOCIETY OF AMERICAN BACTERIOLOGISTS . . . 45 p., illus. Geneva, N. Y. 1926.

In synthetic media isolations Nos. 89 and 95 showed no reduction of nitrates on the second, fourth, ninth, and twenty-first days. At no time was there any reaction for ammonia.

Results of tests for nitrites in beef-extract broth plus 2 parts per million potassium nitrite uninoculated (control) and inoculated with Nos. 89 and 95 were as follows: Second and fourth days, all deep pink; ninth day, control and No. 89 deep pink, but No. 95 a lighter pink than control; and twenty-first day, control deep pink, No. 89 light pink, and No. 95 very light pink.

There is no evidence of gas production in fermentation tubes containing cultures in nitrate-peptone broth.

Since the organism produces ammonia in peptone broth without nitrate, grows only slightly in the synthetic medium, and slowly destroys the nitrite in peptone broth containing 2 parts per million nitrite, the test for nitrate reduction is recorded as inconclusive.

HYDROLYSIS OF STARCH.—There is a partly cleared zone 1 cm. broad around the streak on starch-agar plates after growth for 10 days and 2 cm. broad after 19 days.

FERMENTATION.—Tests were made with sucrose, glucose, lactose, and glycerin, using 2 per cent sugar plus 1 per cent peptone in distilled water, 2 per cent sugar plus 0.2 per cent peptone, 2 per cent sugar plus 0.2 per cent peptone plus 0.5 per cent tartrate, 2 per cent sugar plus 0.5 per cent peptone, and 1 per cent sugar plus ammonium phosphate, as recommended in the Manual of Methods.[7]

No gas was formed in fermentation tubes, and when tested with neutral litmus paper at the end of 16 days the reaction was alkaline in all cultures.

In three tests brom-cresol purple, phenol red, and brom-phenol blue were used as indicators in the media. The cultures were alkaline throughout with dextrose, but with sucrose there was a slight production of acid during the first few days, the reaction later becoming alkaline.

In seven tests quantitative colorimetric pH determinations were made. The results are given in Table 2.

TECHNICAL DESCRIPTION

Bacterium holcicola n. sp.

A motile rod with rounded ends and one to two polar flagella; single, in pairs, or occasionally in short chains; average measurements 0.75μ by 1.58μ; no spores; capsules formed; Gram-negative; not acid fast; stains readily with carbol fuchsine and gentian violet; beef-infusion peptone agar colonies are round, umbonate, glistening, smooth, at first translucent, later more opaque, wax yellow, butyrous, amorphous or with slight radiate markings by transmitted light; gelatin is liquefied slowly; growth in beef-infusion bouillon is moderate and an irregular ring forms at the surface; there is no growth in Cohn's solution (?); slight growth in Uschinsky's solution and light clouding in Fermi's solution and formation of a viscid ring which strings down into the medium; optimum temperature for growth is about 28° to 30° C., maximum 36° to 37°, minimum about 4°; thermal death point 51°; optimum reaction for growth is pH 7.0 to 7.5; the limits of growth, pH 5.5 and pH 9.0; indol is not produced; hydrogen sulphide and ammonia are produced; aerobic; milk is cleared without coagulation; nitrate reduction doubtful; hydrolysis of starch moderate; no gas produced from carbon compounds; a slight amount of acid is formed from sucrose; the reaction with glucose, lactose, and glycerin is alkaline. Pathogenic on leaves of varieties of *Holcus sorghum* and *H. halepensis*.

[7] SOCIETY OF AMERICAN BACTERIOLOGISTS. COMMITTEE ON BACTERIOLOGICAL TECHNIC. Op. cit.

TABLE 2.—*Changes in reaction caused by the growth of Bacterium holcicola in culture solutions containing carbon compounds*

[Quantitative colorimetric determinations were made]

Date inoculated and medium used	Inoculated with culture No.	Incubation period	Hydrogen-ion concentration of medium containing—							
			Sucrose		Glucose		Lactose		Glycerin	
			Inoculated	Uninoculated	Inoculated	Uninoculated	Inoculated	Uninoculated	Inoculated	Uninoculated
		Days								
Nov. 6, 1924: Peptone, 0.2 per cent; carbon compound, 2 per cent	71	2			6.0	5.8				
		4			6.4	5.8				
		7			6.5					
		9			6.6	5.6				
		11			6.7	5.7				
		14			7.0					
		18			7.1	5.7				
		22			7.1	5.6				
Peptone, 0.2 per cent; carbon compound, 2 per cent; tartrate, 0.5 per cent	71	2			6.3	6.1				
		4			6.5	6.1				
		7			6.6					
		9			6.7	6.1				
		11			6.7	6.1				
		14			7.0					
		18			7.1	6.1				
		22			7.2	6.1				
Nov. 26, 1924: Peptone, 0.2 per cent; carbon compound, 2 per cent	72	0		5.8		5.6				
		2	6.1	5.9	5.9	5.6				
		3	5.9	5.8	5.9	5.6				
		5	5.8	5.8	6.0	5.6				
		7	6.1		6.4					
		10	6.4		6.7					
		13	7.0	5.8	6.9	5.5				
		16	7.3		6.9					
		21	7.4	5.9	7.1	5.4				
		31	7.5	6.2	7.6	5.7				
Jan. 3, 1925: Peptone, 0.2 per cent; carbon compound, 2 per cent	72	0				5.8				
		2			5.9					
		3			6.0					
		4			6.1	5.6				
		6			6.3					
		7			6.3	5.8				
Jan. 12, 1925: Peptone, 0.2 per cent; carbon compound, 2 per cent	72	1				5.9				
		2			6.0	5.7				
		4			6.0	5.7				
		7			6.3	5.7				
		9			6.4					
		11			6.8					
Feb. 9, 1925: Ammonium phosphate; carbon compound, 1 per cent	72	0				6.9				
		1			6.8	6.9				
		2			6.8					
		5			6.8					
		7			6.8	6.9				
		8			6.8					
		9			6.7					
		10			6.7					
		12			6.7					
		15			6.7	6.9				
		16			6.7					
		18			6.4					
		19			6.4					
		22			6.4					
		25			6.3	6.8				
		33				6.8				
Nov. 22, 1928: Peptone, 0.5 per cent; carbon compound, 2 per cent	89	1	6.9	7.1	6.6	6.6	6.8	6.9	7.0	7.0
		2	6.8	7.0	6.4	6.5	6.8	6.8	6.9	7.0
		4	6.6	7.0	6.4	6.5	6.8	6.7	7.1	7.1
		8	6.9	7.1	6.8	6.5	7.2	6.8	7.3	7.0
		11	7.1	7.1	7.0	6.4	7.3	6.7	7.2	7.0
		21	7.4	7.1	7.4	6.4	7.6	6.6	7.6	6.9
	95	1	7.0	7.1	6.6	6.6	6.8	6.9	7.0	7.0
		2	6.8	7.0	6.6	6.5	6.8	6.8	7.0	7.0
		4	6.6	7.0	6.5	6.5	6.9	6.7	7.1	7.1
		8	7.0	7.1	6.8	6.5	7.2	6.8	7.3	7.0
		11	7.2	7.1	7.3	6.4	7.5	6.7	7.4	7.0
		21	7.6	7.1	7.7	6.4	7.7	6.6	7.6	6.9

VARIETAL SUSCEPTIBILITY AND MEANS OF DISSEMINATION

The heaviest infection of this disease observed by the writer occurred at Hays, Kans., in 1924.

The varieties listed were grown in a test plot in blocks of 4 to 24 and 48 rows of each variety, each row representing a selection. There were considerable differences in amounts of infection on different plants in the same row, but on the whole they could be listed as follows:

White kafir (C. I.[8] 342) was the only one that appeared to be practically immune, with only an occasional lesion.

White kafir (C. I. 314) and Leoti Red sorgo also showed very little infection.

Sunrise kafir, Progressive kafir, Barchet kaoliang, Early White milo, Buff durra, and a selection of Red Amber × feterita all showed traces of spotting on most of the leaves of all of the plants.

Kafir (C. I. 204), Bishop kafir, Reed kafir, Freed sorgo, Dawn kafir, and Pierce kaferita (H. C.[9] 2523) all showed occasional plants with half of the leaves lightly streaked.

Dwarf feterita, Freed sorgo, Straight-neck milo, Farr's kafir × feterita, and Tricker sorgo all showed a moderate amount of spotting on 50 to 100 per cent of the leaves of all plants in a row.

Pink kafir × milo × feterita, Pink kafir × milo, Dwarf Yellow milo × Dwarf hegari, Wonder kafir, Farr's hegari × milo, Farr's European milo, Farr's Dwarf hegari, and Pink kafir × Dwarf milo all showed heavier spotting than the other varieties, some plants in each showing fairly heavy spotting on all leaves from the lowest to the uppermost.

There were enough lesions on leaves just below the heads to make it quite possible that the seeds might become infected and transmit the disease. To test this, heads from heavily infected plants were collected and the seed sown at the Arlington Experiment Farm, Rosslyn, Va., in May, 1925. Half of each of three lots of seed was treated with uspulun, 0.25 per cent, for one hour, and then washed; and half of each of four lots with formaldehyde, 1:320, for two hours, and washed. The other half of each lot was untreated. Not a single lesion was found on any of these plants throughout the season.

Several attempts to isolate the organism from the seeds have been unsuccessful. The common occurrence of lesions on the second leaf of seedlings points toward either seed or soil transmission.

In 1924 a few water-soaked partly red lesions, like those from Kansas, were found on some milo growing in the cereal plots at the Arlington Experiment Farm. Isolations from these lesions gave typical raised lemon-yellow colonies, but their pathogenicity was not proved. This is the only time the disease has been found at the Arlington farm, and it probably was introduced on the seed.

[8] C. I. indicates a serial number of the Office of Cereal Crops and Diseases.
[9] H. C. indicates a serial number used at Hays, Kans.

COMPARISON WITH OTHER BACTERIAL DISEASES OF SORGHUM

Lesions caused by *Bacterium andropogoni* [10] on sorghum are never water-soaked and translucent, but even the youngest lesions are always red throughout, and no oval spots with tan centers and red borders develop. While lesions caused by *Bact. holcicola* have a reddish cast, there is much more brown in them than in those due to *Bact. andropogoni*. The exudate from lesions due to *Bact. andropogoni* forms red crusts, whereas that from lesions of *Bact. holcicola* forms thin white to cream-colored scales.

The disease caused by *Bacterium holci* Kendrick [11] on species of Holcus is characterized by light-centered, red-bordered, round to elliptical or irregular lesions on the leaves. They vary from 1 to 8 mm. in diameter. Very small lesions are red throughout. They resemble lesions of the disease here described in that at first they are dark green and water-soaked, but the long narrow red streaks of the present disease are neither described nor illustrated by Kendrick.[12]

Pseudomonas alboprecipitans Rosen [13] on Holcus species produces grayish green spots bordered by red bands, or merely by small red irregular spots.

The organism described in this paper differs from the three mentioned above in important cultural, morphological, and physiological characters, which are summarized in Table 3.

TABLE 3.—*Comparative cultural, morphological, and physiological characters of various bacterial organisms which produce lesions on Holcus spp.*

Character compared	Bacterium andropogoni	Bacterium holci	Pseudomonas alboprecipitans	Bacterium holcicola
Color on agar	White	White fluorescent	White	Yellow.
Size (μ)	1.3–2.5 by 0.4–0.8	1.5–2.9 by 0.6–1.0	1.8 by 0.6	1.58 by 0 75
Oxygen relation	Aerobic	Aerobic	Aerobic	Aerobic.
Nitrates reduced	No	Yes	Yes	(?).
Ammonia produced		No	Yes	Yes.
Indol produced	No	No	No	No.
Hydrogen sulphide produced	No		No	Yes.
Acid produced with dextrose	Yes	Yes	No	No
Diastasic action	Yes	No	Yes	Yes.
Thermal death point (°C.)	48°	49°	41°–43°	51°.
Gram, negative or positive	Negative	Negative	Negative	Negative.
Gelatin liquefied	No	Yes	No	Yes.
Milk cleared	Without curd	Without curd	Without curd	Without curd.

SUMMARY

Bacterial streak disease of sorghum is characterized by narrow reddish-brown streaks on the leaves. These lesions are water-soaked in early stages and in later stages may broaden at intervals into elongated oval spots with tan centers and narrow red margins. Exudate is abundant and dries to form thin white to cream-colored scales. Specimens have been collected in Texas, Oklahoma, Kansas, and Montana.

[10] ELLIOTT, C., and SMITH, E. F. A BACTERIAL STRIPE DISEASE OF SORGHUM. Jour. Agr. Research 38: 1-22, illus. 1929.

[11] KENDRICK, J. B. HOLCUS BACTERIAL SPOT OF ZEA MAYS AND HOLCUS SPECIES. Iowa Agr. Expt. Sta. Research Bul. 100, p. 303-334, illus. 1926.

[12] KENDRICK, J. B. Op. cit.

[13] ROSEN, H. R. A BACTERIAL DISEASE OF FOXTAIL (CHAETOCHLOA LUTESCENS). Ann. Missouri Bot. Gard 9: 333-402, illus. 1922.

The disease spreads from younger to older leaves, but apparently it does not check the development of the plant sufficiently to make control measures necessary.

Experimental lesions were produced on Dwarf White milo by spraying with cultures of the organism isolated from young lesions on the same variety collected at Chillicothe, Tex. The organism isolated from lesions on Johnson grass and sprayed on Dwarf White milo after dark has also produced abundant typical infections, thus establishing its pathogenicity.

From the lesions has been isolated a yellow polar-flagellate organism which reproduces the disease when sprayed on healthy plants. This organism, which apparently is undescribed, is given the name *Bacterium holcicola* n. sp.

A study of this organism has been made; its morphology, staining reactions, cultural characters, and physiology are discussed, and a technical description is given.

Some tests of varietal susceptibility of sorghum to the infection were carried out. While the means of dissemination of the infection was not discovered, evidence is adduced to indicate that it spreads by seed or soil transmission. A tabular comparison was made between the cultural, morphological, and physiological characters of *Bacterium holcicola* and those of three other organisms which also attack sorghum, *Bact. holci*, *Bact. andropogoni*, and *Pseudomonas alboprecipitans*.

BEEF EXTRACT AS A SOURCE OF VITAMIN G[1]

By RALPH HOAGLAND, *Senior Biochemist*, and GEORGE G. SNIDER, *Senior Scientific Aid, Biochemic Division, Bureau of Animal Industry, United States Department of Agriculture*

INTRODUCTION

Beef extract is a highly concentrated water extract of fresh lean beef. It consists of those constituents of lean beef which are soluble in water and are not coagulated by heating. In other words, beef extract is concentrated beef broth. As regards nutritive value, beef extract is considered a food adjuvant rather than an important source of nutrients. Beef extract is rich in the flavoring constituents of meat and therefore has important culinary properties. Physiologically it has been found to exert a favorable effect on the flow of digestive fluids. Although 1 pound of solid beef extract contains the water-soluble constituents from 20 or more pounds of lean beef, beef extract has not been found to be a good source of the so-called water-soluble B vitamin. This is in harmony with the fact that lean beef is not well supplied with this vitamin complex.

The recent experiments by Goldberger and his associates[2] which showed that the so-called water-soluble B vitamin really consisted of at least two vitamins—one, the antineuritic, labile to heat, and the other stable to heat and without antineuritic properties—suggested to the writers that commercial beef extract might be a good source of the second vitamin. Goldberger designated the heat-stable vitamin as the P-P factor, meaning pellagra preventive, but in the interest of simplicity in nomenclature a committee of the American Society of Biological Chemists[3] has recommended that the term "vitamin G" be used to donate the more heat-stable, water-soluble dietary factor, and that the term "vitamin B" be restricted to the antineuritic vitamin. The above terminology has been followed in this paper.

The purpose of the experiments reported in this paper was to determine the relative amounts of growth-promoting vitamin G in commercial beef extract obtained from different manufacturers.

METHODS

The relative amounts of vitamin G in samples of beef extract were estimated by feeding tests with young albino rats. Each ration containing a definite proportion of beef extract, calculated in terms of moisture-free extract, was fed to a group of four to six rats which were usually selected from three litters. Each rat was kept in an individual cage with raised screen bottom. The ration was supplied in a self-feeder and an accurate record was kept of feed consumed. Rats weighing approximately 40 grams each and not exceeding 28

[1] Received for publication Jan. 21, 1930, issued May, 1930.

[2] GOLDBERGER, J., WHEELER, G. A., LILLIE, R. D., and ROGERS, L. M. A FURTHER STUDY OF BUTTER, FRESH BEEF, AND YEAST AS PELLAGRA PREVENTIVES, WITH CONSIDERATION OF THE RELATION OF FACTOR P-P OF PELLAGRA (AND BLACK TONGUE OF DOGS) TO VITAMIN B. [U. S.] Pub. Health Rpts. 41: 297-318, illus. 1926.

[3] SEIDELL, A., SHERMAN, H. C., LEVENE, P. A., STEENBOCK, H., MCCOLLUM, E. V., and DUTCHER, R. A. REPORTS. VITAMIN B TERMINOLOGY. Science (n. s.) 69: 276. 1929.

days in age were first fed a basal ration practically free from vitamins B and G until growth ceased. The rats were then fed the test ration consisting of the basal ration supplemented with an alcoholic extract of white corn as the source of vitamin B (antineuritic vitamin) and beef extract as the source of vitamin G. The rats were weighed twice weekly.

Basal ration for rats

Casein (N×6.25)	per cent	20
Ash mixture	do	4
Cod-liver oil	do	2
Hydrogenated cottonseed oil	do	8
Cassava starch to make	do	100

Finely ground commercial casein was thoroughly extracted with 60 per cent alcohol by percolation and then dried. The ash mixture was made up according to a formula by Drummond.[4] The cod-liver oil was a high-grade medicinal product. The hydrogenated cottonseed oil was a well-known commercial product. The cassava starch was a high-grade commercial product.

PREPARATION OF CORN EXTRACT

An alcoholic extract of corn as the source of vitamin B was prepared in a manner similar to that described by Goldberger and his associates.[5] Five kilograms of finely ground white corn were mixed with approximately 12 liters of 85 per cent by volume of ethyl alcohol. The mixture was stirred at intervals, allowed to stand overnight, and then transferred to percolators. The filtrate was concentrated on a steam bath with the aid of a blast of air directed over the surface of the liquid. When most of the alcohol had evaporated and protein was precipitating from solution, the dish was removed from the steam bath and 650 grams of cassava starch was mixed with the concentrated extract. The mixture was spread out in a thin layer in shallow pans and dried at 60° C. or lower in an oven with a forced draft. The dried material was ground fine and stored in covered jars. Five kilograms of corn meal yielded approximately 730 grams of dried extract; hence 1 gram of extract corresponded to approximately 6.8 grams of corn.

DESCRIPTION OF BEEF EXTRACT

Pure concentrated beef extract was obtained from five meat-packing establishments operating under Federal inspection. For purposes of identification these establishments will be designated by the letters V, W, X, Y, and Z. Each lot of extract was first dissolved in water in order to facilitate mixing with the other constituents of the ration. The proportion of solids in the diluted extract was determined, and a sufficient quantity to furnish the desired proportion of extract in the ration was mixed with the calculated amount of starch. The mixture was dried and combined with the other constituents of the ration.

[4] DRUMMOND, J. C., and COWARD, K. H. RESEARCHES ON THE FAT-SOLUBLE ACCESSORY SUBSTANCE. V. THE NUTRITIVE VALUE OF ANIMAL AND VEGETABLE OILS AND FATS CONSIDERED IN RELATION TO THEIR COLOUR. Biochem. Jour. 14: [668]–677. 1920.

[5] GOLDBERGER, J., WHEELER, G. A., LILLIE, R. D., and ROGERS, L. M. Op. cit.

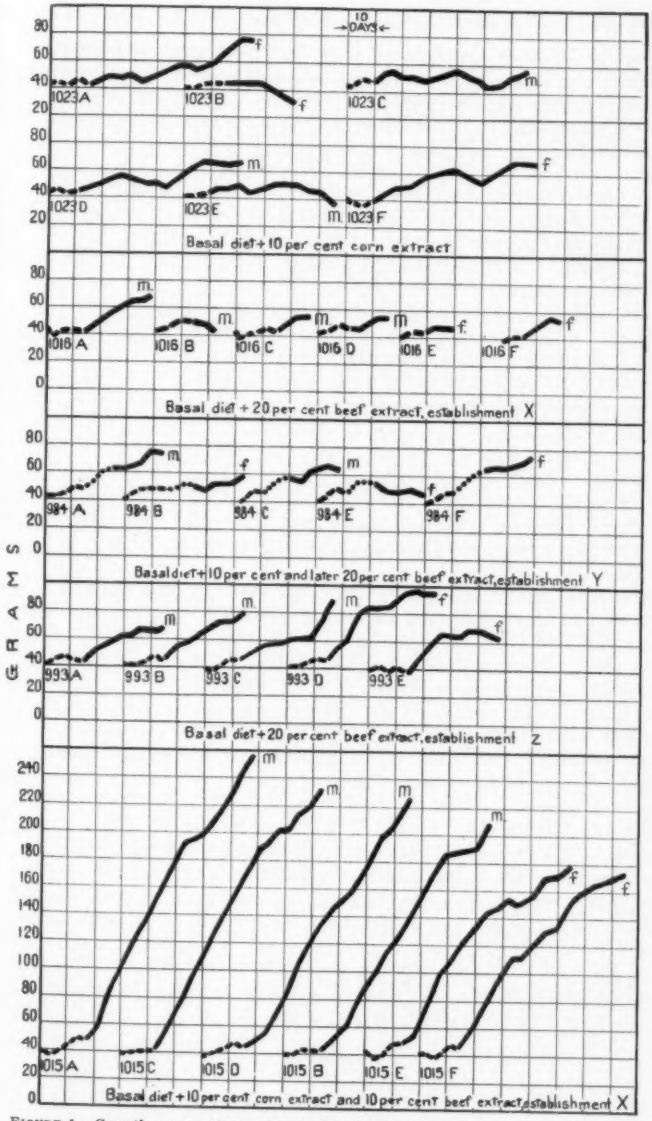

FIGURE 1.—Growth curves of rats showing effects of a deficiency of vitamins B and G, respectively, on growth. The broken lines indicate results of preliminary feeding on a diet very deficient in both vitamins B and G. The letters *m* and *f* denote sex. Rats Nos. 1023A to 1023F, inclusive, were fed a diet very deficient in vitamin G; rats Nos. 1016A to 1016F, 984A to 984F, and 993A to 993E, inclusive, were fed diets very deficient in vitamin B, while rats Nos. 1015A to 1015F, inclusive, were fed diets containing adequate amounts of both vitamins B and G

RESULTS OF EXPERIMENTS

The basal ration supplemented with 10 per cent of corn extract as the source of vitamin B (antineuritic) was tested at intervals during these experiments to determine whether an appreciable amount of vitamin G was present. In all these tests the rats made but slight, if any, growth, indicating that the ration contained only a very small amount of this vitamin. On the other hand, when the ration was supplemented with autoclaved yeast as the source of vitamin G, the rats made excellent growth. These results show that the corn extract supplied an adequate amount of vitamin B for growth.

In Figure 1 the first group of curves (Nos. 1023A to 1023F, inclusive) shows the results obtained by feeding rats a ration adequate for normal growth except for lack of vitamin G. The rats were first fed the basal ration lacking both vitamins B and G until growth ceased (broken lines) when corn extract was added to the ration as a source of vitamin B (continuous lines). The failure of these rats to grow indicates that the ration did not contain an appreciable amount of vitamin G.

Three groups of rats—Nos. 1016, 984, and 993 (fig. 1)—were fed rations containing 20 per cent each of beef extract from establishments X, Y, and Z, respectively, as the sole addition to the basal ration. The slight growth made by these rats indicated that these samples of beef extract were very deficient in either vitamin B or vitamin G, or both.

Rats Nos. 1015A to 1015F, inclusive, the growth curves of which are shown at the bottom of Figure 1, were fed a ration containing 10 per cent of beef extract from establishment X as the source of vitamin G, the diet being adequate in other respects for normal growth. The excellent growth made by these rats indicates that 10 per cent of this lot of beef extract supplied an ample quantity of vitamin G. It is apparent, then, that the factor which limited the growth of rats Nos. 1016A to 1016F, inclusive, which received 20 per cent of the same lot of beef extract as the source of both vitamins B and G, was lack of vitamin B alone. Evidence to be presented later will show that lack of vitamin B was also the limiting factor in the growth of rats Nos. 984A to 984F, inclusive, and rats Nos. 993A to 993E, inclusive.

In Figures 2 to 6, inclusive, are shown the growth curves of rats which were fed rations containing beef extract from establishments V, W, X, Y, and Z, respectively, as the source of vitamin G. Beef extract from each establishment was fed at three levels of intake, viz, 5, 7.5, and 10 per cent, the percentage being calculated in terms of moisture-free extract. In Table 1 are presented data concerning the growth and feed consumption of these rats.

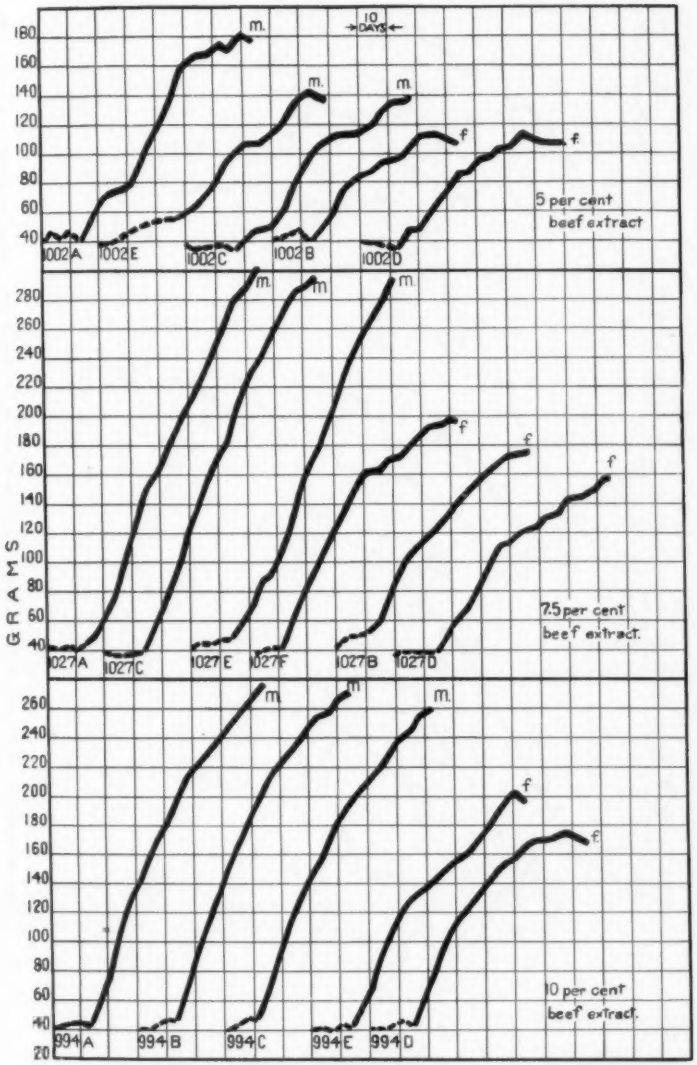

FIGURE 2.—Growth curves of rats fed diets containing beef extract from establishment V as the source of vitamin G

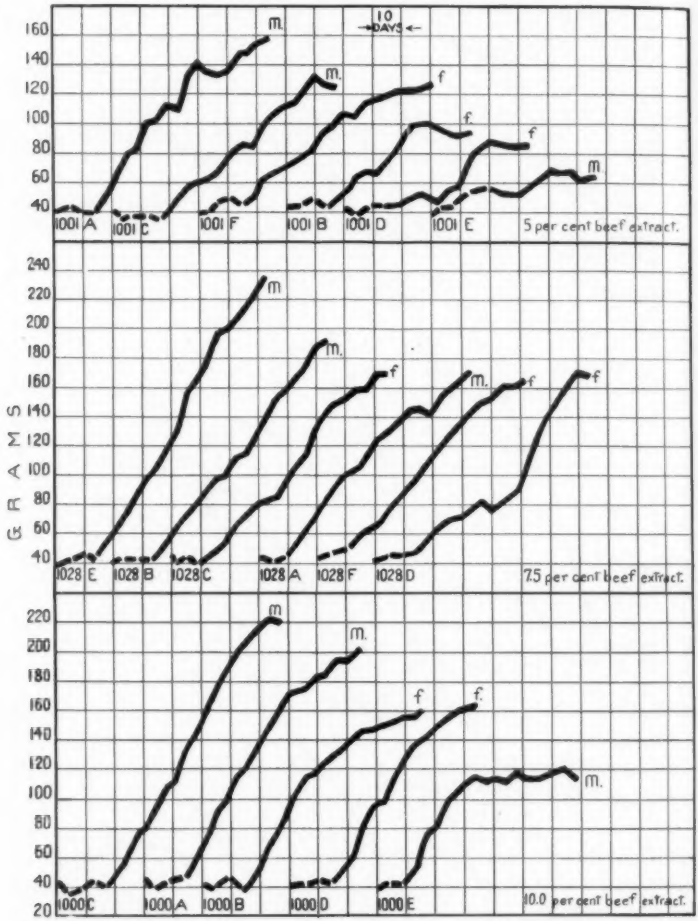

FIGURE 3.—Growth curves of rats fed diets containing beef extract from establishment W as the source of vitamin G

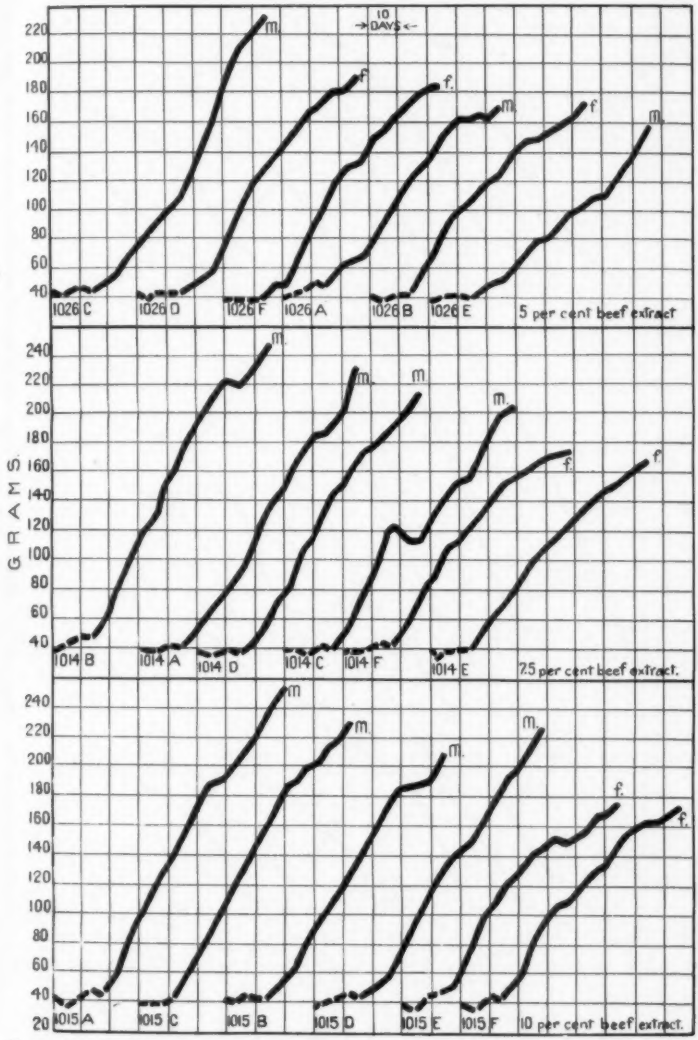

FIGURE 4.—Growth curves of rats fed diets containing beef extract from establishment X as the source of vitamin G

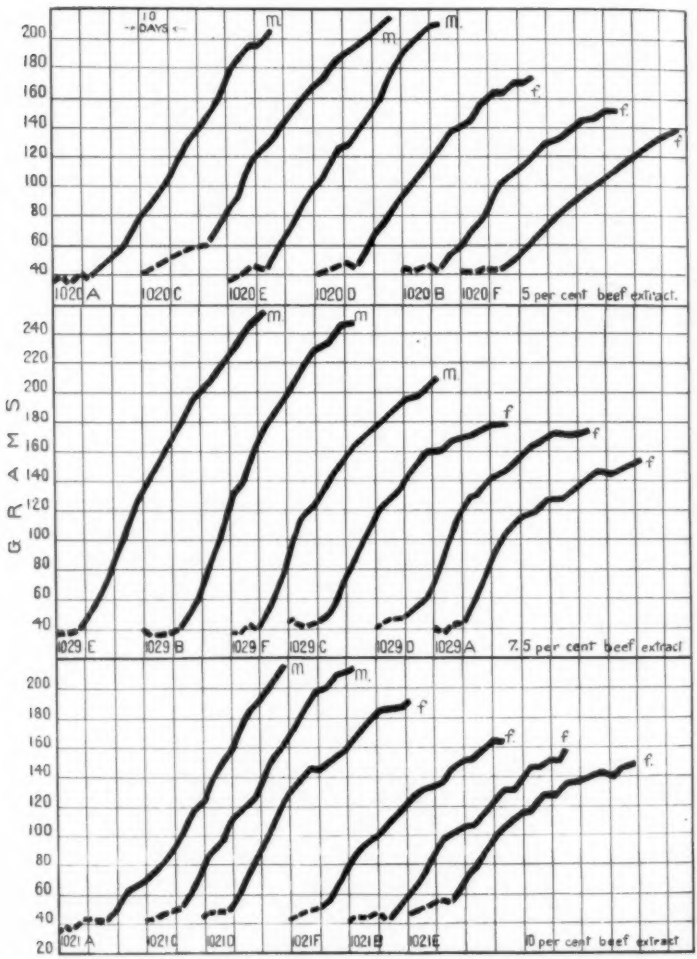

FIGURE 5.—Growth curves of rats fed diets containing beef extract from establishment Y as the source of vitamin G

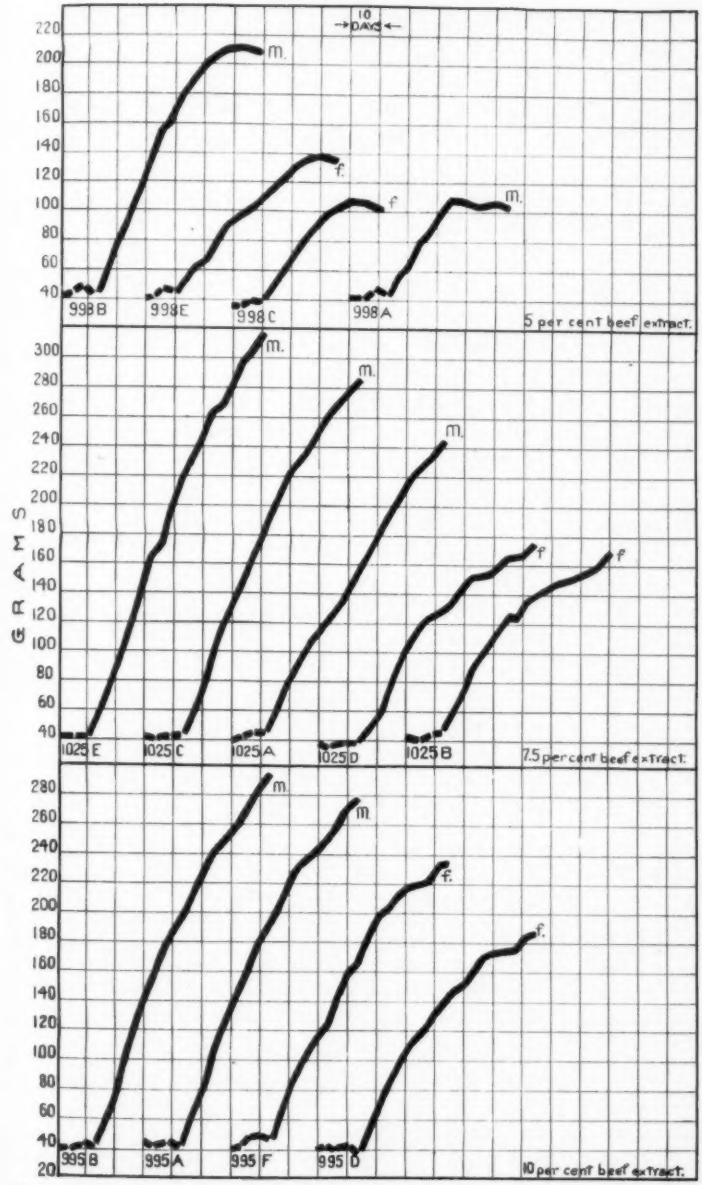

FIGURE 6.—Growth curves of rats fed diets containing beef extract from establishment Z as the source of vitamin G

TABLE 1.—*Feed consumed and gains made by rats fed different quantities of beef extract as a source of vitamin G*

Addition to basal ration	Rat No.	Sex	Duration of test	Total gain in weight	Average daily gain in weight	Total feed consumed	Total beef extract consumed	Average daily intake of beef extract	Average gain in weight per gram of feed eaten
			Days	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Gram*
5 per cent beef extract (establishment V).	1002A	Male	60	137	2.28	517	25.85	0.43	0.265
	1002B	Female	49	69	1.41	319	15.95	.33	.216
	1002C	Male	60	104	1.73	384	19.20	.32	.271
	1002D	Female	56	73	1.30	332	16.60	.30	.220
	1002E	Male	52	82	1.58	375	18.75	.36	.219
7.5 per cent beef extract (establishment V).	1027A	do	60	258	4.30	721	54.08	.90	.358
	1027B	Female	60	125	2.08	516	38.70	.65	.242
	1027C	Male	60	257	4.28	780	58.50	.98	.329
	1027D	Female	60	121	2.02	471	35.33	.59	.257
	1027E	Male	56	246	4.39	665	49.88	.89	.370
	1027F	Female	60	158	2.63	607	45.53	.76	.260
10 per cent beef extract (establishment V).	994A	Male	60	233	3.88	577	57.70	.96	.404
	994B	do	60	224	3.73	645	64.50	1.08	.347
	994C	do	60	214	3.57	633	63.30	1.06	.338
	994D	Female	60	126	2.10	529	52.90	.88	.238
	994E	do	60	156	2.60	540	54.00	.90	.289
5 per cent beef extract (establishment W).	1001A	Male	60	119	1.98	517	25.85	.43	.230
	1001B	Female	49	51	1.04	294	14.70	.30	.173
	1001C	Male	60	92	1.53	422	21.10	.35	.218
	1001D	Female	49	43	.88	217	10.85	.22	.198
	1001E	Male	38	8	.21	158	7.90	.21	.051
	1001F	Female	62	79	1.27	436	21.80	.35	.081
7.5 per cent beef extract (establishment W).	1028A	Male	62	125	2.02	502	37.65	.61	.249
	1028B	do	62	151	2.44	503	37.73	.61	.300
	1028C	Female	62	130	2.10	522	39.15	.63	.249
	1028D	do	62	123	1.98	435	32.63	.53	.283
	1028E	Male	60	199	3.32	594	44.55	.74	.335
	1028F	Female	60	116	1.93	481	36.08	.60	.241
10 per cent beef extract (establishment W).	1000A	Male	60	156	2.60	583	58.30	.97	.268
	1000B	Female	60	123	2.05	530	53.00	.88	.232
	1000C	Male	60	181	3.02	592	59.20	.99	.306
	1000D	Female	49	121	2.47	415	41.50	.85	.292
	1000E	Male	60	71	1.18	373	37.30	.62	.190
5 per cent beef extract (establishment X).	1026A	do	60	122	2.03	499	24.95	.42	.244
	1026B	Female	63	132	2.10	528	26.40	.42	.250
	1026C	Male	60	188	3.13	575	28.75	.48	.327
	1026D	Female	60	149	2.48	561	28.05	.47	.266
	1026E	Male	60	117	1.95	377	18.85	.31	.310
	1026F	Female	60	147	2.45	550	27.50	.46	.267
7.5 per cent beef extract (establishment X).	1014A	Male	60	190	3.17	519	38.93	.65	.366
	1014B	do	60	201	3.35	677	50.78	.85	.297
	1014C	do	61	166	2.72	583	43.73	.72	.285
	1014D	do	60	177	2.95	528	39.60	.66	.335
	1014E	Female	60	128	2.13	475	35.63	.59	.269
	1014F	do	60	129	2.15	476	35.70	.60	.271
10 per cent beef extract (establishment X).	1015A	Male	61	210	3.44	634	63.40	1.04	.331
	1015B	do	60	169	2.82	448	44.80	.75	.377
	1015C	do	60	190	3.17	600	60.00	1.00	.317
	1015D	do	61	183	3.00	527	52.70	.86	.347
	1015E	Female	60	130	2.17	498	49.80	.83	.261
	1015F	do	61	129	2.11	498	49.80	.82	.259
5 per cent beef extract (establishment Y).	1020A	Male	61	166	2.72	535	26.75	.44	.310
	1020B	Female	61	108	1.77	484	24.20	.40	.223
	1020C	Male	63	148	2.35	621	31.05	.49	.238
	1020D	Female	60	127	2.12	512	25.60	.43	.248
	1020E	Male	60	165	2.75	543	27.15	.45	.304
	1020F	Female	60	95	1.58	380	19.00	.32	.250
7.5 per cent beef extract (establishment Y).	1029A	do	60	113	1.88	550	41.25	.69	.205
	1029B	Male	62	207	3.34	662	49.65	.80	.313
	1029C	Female	62	135	2.18	573	42.98	.69	.236
	1029D	do	62	128	2.06	585	43.88	.71	.219
	1029E	Male	62	216	3.48	693	51.98	.84	.312
	1029F	do	60	167	2.78	642	48.15	.80	.260
10 per cent beef extract (establishment Y).	1021A	do	62	173	2.79	529	52.90	.85	.327
	1021B	Female	60	116	1.93	443	44.30	.74	.262
	1021C	Male	60	165	2.75	563	56.30	.94	.293
	1021D	Female	62	143	2.31	579	57.90	.93	.247
	1021E	do	63	95	1.51	481	48.10	.76	.198
	1021F	do	62	111	1.79	526	52.60	.85	.211
5 per cent beef extract (establishment Z).	998A	Male	42	59	1.40	242	12.10	.29	.244
	998B	do	56	166	2.96	531	26.55	.47	.313
	998C	Female	42	63	1.50	230	11.50	.27	.274
	998E	do	56	93	1.66	359	17.95	.32	.259

TABLE 1.—*Feed consumed and gains made by rats fed different quantities of beef extract as a source of vitamin G*—Continued

Addition to basal ration	Rat No.	Sex	Duration of test	Total gain in weight	Average daily gain in weight	Total feed consumed	Total beef extract consumed	Average daily intake of beef extract	Average gain in weight per gram of feed eaten
			Days	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Gram*
7.5 per cent beef extract (establishment Z).	1025A	Male	60	198	3.30	622	46.65	0.78	0.318
	1025B	Female	60	126	2.10	550	41.25	.69	.229
	1025C	Male	60	242	4.03	773	57.98	.97	.313
	1025D	Female	60	136	2.27	504	37.80	.63	.273
	1025E	Male	60	277	4.62	831	62.33	1.04	.333
10 per cent beef extract (establishment Z).	995A	do	60	236	3.93	660	66.00	1.10	.358
	995B	do	60	251	4.18	673	67.30	1.12	.373
	995D	Female	60	148	2.47	501	50.10	.84	.295
	995F	do	60	165	2.75	566	56.60	.94	.292

Referring to the growth curves at the top of each of Figures 2 to 6, inclusive, one may observe that 5 per cent of beef extract from establishments X and Y, respectively (figs. 4, 5), furnished sufficient vitamin G for good but not for normal growth. On the other hand, the same proportion of beef extract from establishments V, W, and Z, respectively (figs. 2, 3, 6), induced only poor to fair growth.

From Table 1 it appears that the three male rats which were fed 5 per cent of beef extract from establishment X (fig. 4) made an average daily gain of 2.37 grams and the three female rats a gain of 2.34 grams. The average daily consumption of beef extract by the male rats was 0.40 gram and by the female rats 0.45 gram.

Five per cent of beef extract from establishment Y induced an average daily gain of 2.60 grams in three male rats and 1.82 grams in three female rats. The average daily intake of beef extract by the male rats was 0.46 gram and by the female rats 0.38 gram.

Seven and one-half per cent of beef extract from establishments V, X, Y, and Z, respectively, promoted good to excellent growth in rats (figs. 2, 4, 5, and 6), while the same proportion of extract from establishment W (fig. 3) induced only fair growth. Some of the male rats made extraordinary growth. (Figs. 2, 6.)

From Table 1 the average daily gains made by the male rats fed rations containing 7.5 per cent of beef extract from establishments V, W, X, Y, and Z, respectively, were calculated to be as follows: 4.32, 2.59, 3.05, 3.20, and 3.98 grams. The average daily gains made by the female rats fed the same rations were as follows: 2.24, 2.00, 2.14, 2.04, and 2.19 grams, respectively. The average daily consumption of beef extract by the male rats which were fed rations containing 7.5 per cent beef extract from the above-named establishments was as follows: 0.92, 0.65, 0.72, 0.81, and 0.93 gram, respectively. The average daily consumption of beef extract by the female rats receiving the same rations was as follows: 0.67, 0.59, 0.60, 0.70, and 0.66 gram, respectively.

The data for the rats fed rations containing 10 per cent of beef extract need not be discussed in detail. An examination of Figures 2 to 6, inclusive, shows that the rats fed this proportion of beef extract did not, on an average, make more rapid growth than those receiving 7.5 per cent of beef extract from the same establishment.

Reference was previously made to the very poor growth made by three groups of rats fed rations containing 20 per cent each of beef extract from establishments X, Y, and Z, respectively, as the source of both vitamins B and G (fig. 1, rats 1016A to 1016F, 984A to 984F,

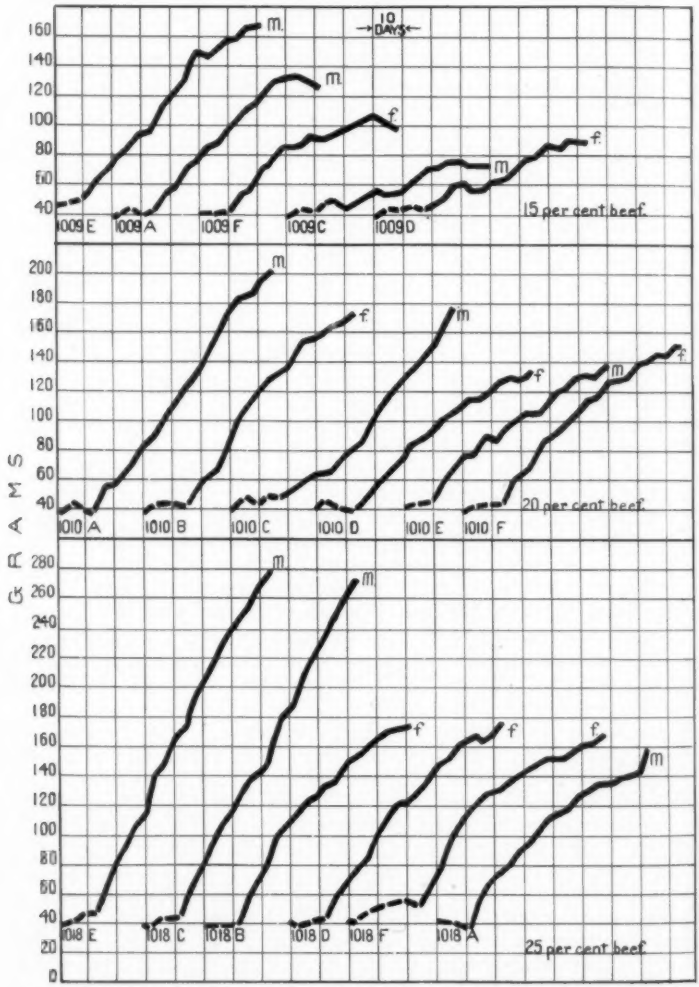

FIGURE 7.—Growth curves of rats fed diets containing dried lean beef as the source of vitamin G

and 993A to 993E). Evidence was presented which showed that the very poor growth made by the rats fed beef extract from establishment X was due to a deficiency of vitamin B. On referring to Figures 5 and 6, one will note that rats fed rations containing 7.5 per cent of beef extract from establishments Y and Z, respectively, as the source of

vitamin G, the ration containing an adequate amount of vitamin B, made normal growth. Hence, the very poor growth made by the rats getting 20 per cent of beef extract from these establishments as the source of both vitamins B and G was due to a deficiency of vitamin B.

VITAMIN G IN BEEF

For the purpose of comparing the amount of vitamin G in beef extract with that in lean beef, there are shown in Figure 7 the growth curves of rats fed different proportions of dried lean beef as a source of this vitamin. These graphs indicate that neither 15 nor 20 per cent of dried beef furnished sufficient vitamin G for normal growth. Twenty-five per cent of beef induced excellent growth in all but one rat. In Table 2 are presented data concerning the growth and feed intake of these rats.

TABLE 2.—*Record of gains made by rats fed different quantities of lean beef as a source of vitamin G*

Addition to basal ration	Rat No.	Sex	Duration of test	Total gain in weight	Average daily gain in weight	Total feed consumed	Total beef consumed	Average daily intake of beef	Average gain per gram of feed eaten
			Days	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Gram*
15 per cent dried lean beef.	1009A	Male	56	84	1.50	425	63.75	1.14	0.198
	1009C	do	57	23	.40	244	36.60	.64	.094
	1009D	Female	56	47	.84	280	42.00	.75	.168
	1009E	Male	60	117	1.95	546	81.90	1.37	.214
	1009F	Female	56	54	.96	382	57.30	1.02	.141
20 per cent dried lean beef.	1010A	Male	60	166	2.77	524	104.80	1.75	.317
	1010B	Female	60	128	2.13	529	105.80	1.76	.242
	1010C	Male	60	129	2.15	426	85.20	1.42	.303
	1010D	Female	60	93	1.55	444	88.80	1.48	.209
	1010E	Male	60	93	1.55	416	83.20	1.39	.224
	1010F	Female	60	108	1.80	471	94.20	1.57	.229
25 per cent dried lean beef.	1018A	Male	60	120	2.00	513	128.25	2.14	.234
	1018B	Female	60	136	2.27	532	133.00	2.22	.256
	1018C	Male	60	224	3.73	688	172.00	2.87	.326
	1018D	Female	60	134	2.23	492	123.00	2.05	.272
	1018E	Male	60	231	3.85	747	186.75	3.11	.309
	1018F	Female	63	116	1.84	559	139.75	2.22	.208

The male rats receiving 25 per cent of dried beef made an average daily gain of 3.19 grams and the female rats an average gain of 2.11 grams. The average daily consumption of dried beef by the male rats was 2.71 grams and by the female rats 2.16 grams. For comparison, the average daily consumption of beef extract by the male rats receiving 7.5 per cent of extract in their diet was 0.80 gram and by the female rats 0.65 gram. These data indicate that the daily intake of dried beef necessary to furnish sufficient vitamin G for normal growth in male or female rats was approximately three and four-tenths times as large as the required amount of beef extract. These comparisons are made between moisture-free beef extract and air-dry beef. If the comparison is made between commercial beef extract containing approximately 20 per cent of moisture and fresh lean beef with a moisture content of 75 per cent, then 1 part of beef extract appeared to contain approximately as much vitamin G as 11 parts of lean beef.

DISCUSSION OF RESULTS

As regards the antipellagric potency of beef extract, the writers have no direct information, but the following comparison may throw some light on the subject. Goldberger and Tanner[6] found that dried yeast in daily doses of 30 grams was very effective in preventing pellagra in human subjects for at least a year. In later experiments with yeast extract, Goldberger and his associates[7] found that a daily dose of 15 grams of this product was as effective in preventing pellagra as 30 grams of yeast. The yeast extract was simply a dried, water-extract of yeast. Goldberger and his associates have also tested the pellagra-preventive action of fresh lean beef. The daily administration of 200 grams of fresh lean beef, which corresponds to approximately 50 grams of moisture-free beef, fully protected human subjects against pellagra for at least a year. Since yeast extract has been found to be much more effective in preventing pellagra than yeast, it may be that beef extract will likewise be more effective against pellagra than beef, comparisons being made between moisture-free products.

SUMMARY

Experiments were conducted with albino rats to determine the relative amounts of vitamin G in commercial beef extract obtained from five manufacturers. For comparison fresh lean beef was examined also.

It was found that an intake level of 7.5 per cent of moisture-free beef extract from each of four establishments furnished sufficient vitamin G for good to excellent growth in rats. The same percentage of beef extract from the other establishment promoted fair growth. In these experiments the average daily intake of moisture-free beef extract was approximately 0.80 gram for males and 0.65 gram for females.

Twenty per cent of dried lean beef furnished sufficient vitamin G for fair growth and 25 per cent enough for excellent growth in rats. The average daily intake of 25 per cent dried beef amounted to 2.7 grams for male and 2.2 grams for female rats.

One pound of moisture-free beef extract appeared to contain approximate'y the same amount of vitamin G as 3.4 pounds of dried beef. If the commercial products are compared, then 1 pound of concentrated beef extract contained approximately the same amount of vitamin G as 11 pounds of fresh, lean beef.

[6] GOLDBERGER, J., and TANNER, W. F. A STUDY OF THE PELLAGRA-PREVENTIVE ACTION OF DRIED BEANS, CASEIN, DRIED MILK, AND BREWERS' YEAST WITH A CONSIDERATION OF THE ESSENTIAL PREVENTIVE FACTORS INVOLVED. [U. S.] Pub. Health Rpts. 40: 54-80. 1925.

[7] GOLDBERGER, J., WHEELER, G. A., LILLIE, R. D., and ROGERS, L. M. Op. cit.

CAROTENOSIS OF BOVINE LIVERS ASSOCIATED WITH PARENCHYMATOUS DEGENERATION [1]

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INTRODUCTION

In the course of post-mortem inspection at slaughtering establishments operating under Federal meat inspection, various pathological conditions in bovine livers are observed by veterinary inspectors. Most of these conditions are of common occurrence, are recognized quite readily, and disposition in such cases can usually be made on the macroscopic appearance of the liver lesions. It occasionally happens, however, that a condition is observed which is immediately recognized as something out of the ordinary but can not be definitely diagnosed by gross examination of the affected liver.

During the last year the attention of the Meat Inspection Division was directed to an unusual condition in the livers of cattle, the outstanding gross characteristic of which was an intense yellow or reddish-yellow coloration of the liver tissue, while all other organs and tissues in the carcass, except the associated hepatic lymph gland which showed a yellowish mottled appearance, were normal in color and general appearance.

In cooperation with the Meat Inspection Division, the Pathological Division undertook an investigation of the liver condition in order to determine, if possible, the cause of the coloration. A request was sent to a number of inspectors in charge of slaughtering establishments for specimens of the yellow livers.

On receipt of the first lot of specimens, one of the writers (Creech) recalled that several livers similar in appearance had been received at the pathological laboratory a number of years previously. The laboratory records showed that the lot of yellow livers referred to had been sent to the laboratory in 1920 by J. S. Jenison, inspector in charge, National Stock Yards, Ill., for a determination of the cause of the yellow color.

The histological findings in the specimens from Jenison were "well-marked parenchymatous degeneration with round-cell infiltration and limited fibrous proliferation." Chemical studies of this lot of livers were made at the same time by W. N. Berg, formerly of the Bureau of Animal Industry. He found the pigmentation, or yellow coloration, to be due to "a substance identical with carotin." When these findings were made the condition was considered as being somewhat rare, and no further studies of yellow livers were made at that time.

Specimens of the yellow livers studied during the present investigations were received from five cattle-slaughtering centers, viz,

[1] Received for publication Jan. 15, 1930: issued May, 1930.

Chicago, Ill., Fort Worth, Tex., Kansas City, Kans., Buffalo, N. Y., and Phoenix, Ariz.

GROSS APPEARANCE OF AFFECTED LIVERS

The typical yellow livers have a characteristic appearance and should not be confused with livers showing other off-color conditions seen more frequently, such as bile discoloration in icteric conditions or yellowish, fatty livers. The affected livers are usually slightly enlarged, with well-rounded borders. In the early stages of the conditions the one outstanding characteristic, and in some cases practically the only deviation from the normal that can be detected in the gross specimen, is the intense yellow or reddish-yellow color of the liver tissue.

When fresh specimens of these livers are sectioned and handled, the knife and fingers are stained a deep yellow, which is distinctly different from the greenish-yellow tinge in bile-pigment staining.

As the pathological changes become more advanced the livers undergo fibrous changes. It has been observed that these fibrous proliferations may vary in the different liver specimens from a sprinkling of slight, fibrous areas barely visible in the gross specimen to advanced cirrhosis involving a large portion, possibly from one-third to one-half of the organ. The extreme cirrhotic changes are usually accompanied by more or less calcification

In all cases examined in which the associated hepatic lymph glands accompanied the liver specimen, these glands exhibited a peculiar, yellowish, mottled appearance. The unusual deep-yellow color, with fibrous changes in some cases, as already indicated, and the lack of definite knowledge as to the cause of the condition, have led to the application of various terms, more or less descriptive, such as icteric liver, lutein liver, alkali liver, and Pictou disease, in designating this type of liver.

It appears that the condition is not confined to any particular age or sex. Some of the livers examined were from steers 2 to 3 years of age while others were from cows possibly 5 years old or older. The condition has been observed also in livers of aged range bulls.

It is of particular interest to note that no other abnormal changes are seen in any other organs or tissue of carcasses in which the yellow livers are found, with the exception, as previously indicated, of the hepatic gland. The fat and other tissue do not show any yellowish pigmentation, thus indicating that in all probability the condition is confined to the liver and associated lymph gland.

The history obtained thus far regarding the origin of cattle with yellow livers indicates that the condition is found most frequently, if not altogether, in cattle from the southwestern part of the United States.

CAUSE OF THE YELLOW PIGMENTATION

The close similarity between the livers examined in the present study and the specimens of yellow livers observed in 1920, in which the intense yellow color was found to be due to a substance identical with carotene, led to the belief that this was the same liver condition. It was decided, therefore, to make a chemical analysis of a number of specimens to identify definitely the coloring matter present.

CHEMICAL STUDIES

It was considered necessary to reinvestigate the coloring matter present in these livers because Berg had not published the evidence on which he based his conclusion that the substance was carotene; moreover, it was not certain that the more recent condition was the same as that studied by him. The coloring matter, therefore, was isolated in quantity and identified by chemical methods.

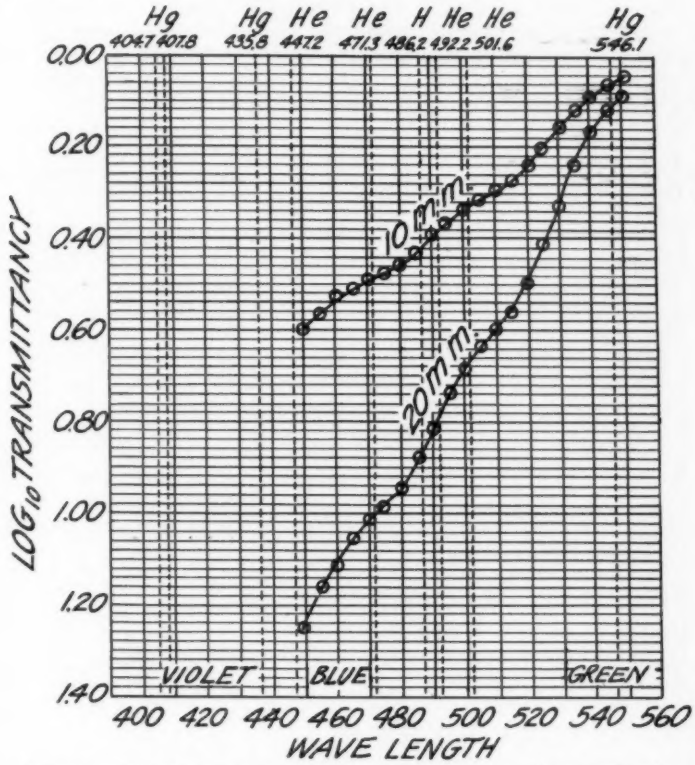


FIGURE 1.—Spectrophotometric measurements of a 0.05 per cent solution of carotene in carbon disulphide

A composite sample of livers 1, 2, and 3, amounting to 4,110 gm., was finely minced in a meat chopper, placed in a 12-liter flask, and 4 liters of 5 per cent alcoholic potash were added. The whole was boiled four hours under reflux, when nearly all the liver tissue was liquefied. The product was strained through cheesecloth and the alcohol removed by distillation. When cold the aqueous residue was extracted with successive portions of petroleum ether until that solvent was no longer deeply colored. The combined petroleum-ether solutions were washed with 85 per cent alcohol to remove xanthophyll, if present, and other possible impurities, and then the petroleum ether

was removed by distillation. The residue was a blood-red, thick, unctuous mass. This was purified by boiling with 5 per cent alcoholic potash, removing the alcohol, extracting with petroleum ether, washing with 85 per cent alcohol, and distilling off the petroleum ether. The residue was dissolved in carbon disulphide and the solution poured into 5 volumes of alcohol. The precipitated carotene was collected, washed with alcohol, and warmed on a water bath to remove volatile impurities.

The substance so obtained was identified as carotene as follows: It responded to the ferric chloride and sulphuric acid color tests; it showed the solubility relationships characteristic of carotene; the triiodide prepared according to the method of Arnaud[2] melted at 138°–139° without decomposition. Palmer[3] gives the melting point 136°–137°, which is sufficiently close for identification. Spectrophotometric measurements of a 0.05 per cent solution of the substance, made at the Bureau of Standards, United States Department of Commerce, are shown in Figure 1, through the courtesy of K. S. Gibson, of that bureau.

A number of determinations of the quantity of carotene present in yellow livers submitted for study, and in normal livers obtained from a local packing plant, were made. In these determinations the excellent method of Connor[4] was used. The results are given in Table 1.

TABLE 1.—*Carotene content and condition of affected and normal livers*

CATTLE LIVERS

Date	Animal	Carotene content	Condition of liver
1928		*Mgm. per 100 gm.*	
Sept. 24	Steer 1	30.4	Yellow.
Do.	Steer 2	45.1	Do.
Do.	Steer 3	29.6	Do.
Sept. 26	Steer	1.3	Normal.
Do.	do.	1.01	Do.
Do.	do.	.99	Do.
Nov. 5	Steer 4	49.8	Yellow.
Do.	Steer 5	21.08	Do.
Do.	Steer 6	7.2	Cirrhotic.
Do.	Steer 7	6.43	Yellow.
Dec. 22	Cow 1	18.9	Do.
Do.	Cow 2	43.4	Do.
1929			
Feb. 14	Steer 8	16.6	Do.
Apr. 19	Steer 9	4.0	Do.

LIVERS OF RATS AFTER FEEDING ON TYPICAL YELLOW LIVERS

1928			
Oct. 31	Rat 1	0.48	Pale, with necrotic areas.
Do.	Rat 2	.8	Do.
Do.	Rat 3	(a)	Do.
Do.	Rat (control)	(a)	Normal.

a Trace.

[2] ARNAUD, A. RECHERCHES SUR LA COMPOSITION DE LA CAROTINE, SA FONCTION CHIMIQUE ET SA FORMULE. Compt. Rend. Acad. Sc. [Paris] 102: 1119–1122. 1886.

[3] PALMER, L. S. CAROTINOIDS AND RELATED PIGMENTS; THE CHROMOLIPOIDES. p. 234. New York. 1922.

[4] CONNOR, C. L. STUDIES ON LIPOCHROMES. III. THE QUANTITATIVE ESTIMATION OF CAROTIN IN BLOOD AND TISSUES. Jour. Biol. Chem. 77: 619–626. 1928.

In all cases search was made for other lipochromes, particularly xanthophyll, but none were found. Since carotene itself is not a toxic substance,[5] further study is needed to determine what deleterious compound is present in these livers. The investigation is being continued in the Bureau of Animal Industry.

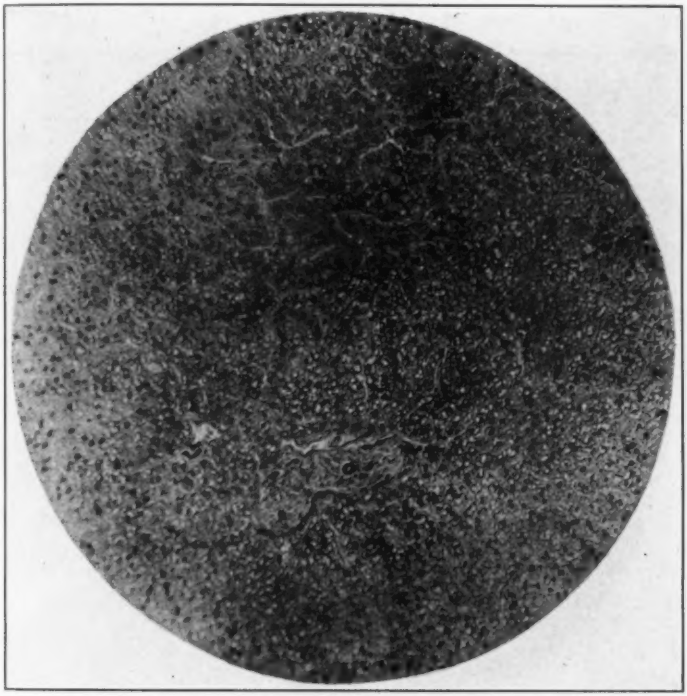


FIGURE 2.—Photomicrograph of section of liver of cow No. 2, showing degeneration of the liver cells. Many of the cells contain fat droplets. × 120

HISTOPATHOLOGY

In the earlier stages of carotenosis of the liver the outstanding pathological change is that of a parenchymatous degeneration. The degenerative changes may vary in different parts of the same liver from a slight cloudiness, or granular appearance of the cell protoplasm, to complete degeneration and disintegration of groups of liver cells. In the well-advanced cases these areas of degeneration and necrosis are more extensive, including whole lobules or groups of lobules.

[5] WELLS, H. G., and HEDENBURG, O. F. THE TOXICITY OF CAROTIN. Jour. Biol. Chem. 27: 213–216. 1916.

The degenerative changes appear to have their beginning most frequently in the central portion of the lobules. There is a very noticeable vacuolation of the liver cells in the degenerated areas, and frequently the cells, particularly toward the periphery of the affected lobules, contain numerous fat droplets. (Figs. 2 and 3.)

In a few of the cases examined practically no normal liver cells could be found in any of the sections made from various parts of the

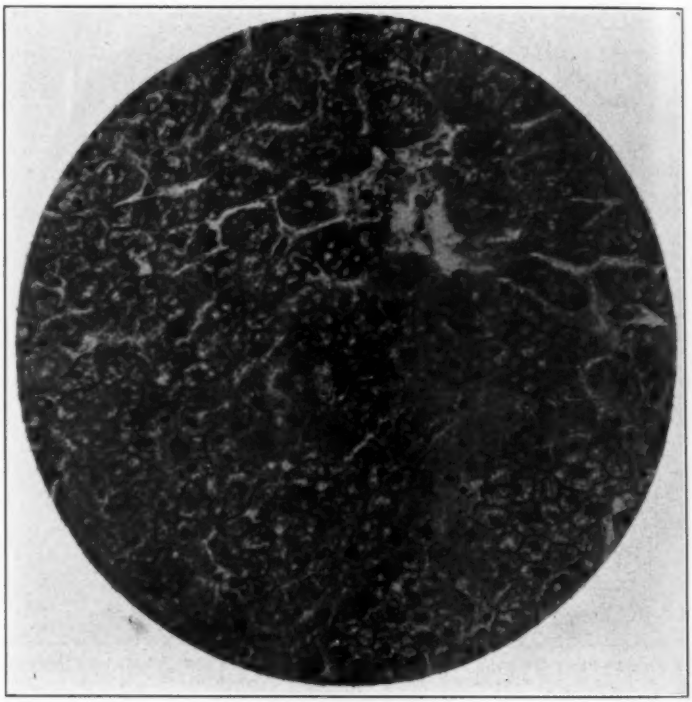


FIGURE 3.—Photomicrograph of section of liver of steer No. 1, showing extensive degeneration of the cells. × 620

specimens. (Fig. 4.) Some specimens show an engorgement of the central veins and capillaries with small hemorrhages here and there.

Areas of round-cell infiltration are frequently seen. These are the first indication of the fibrous changes which occur in the later stages of the disease. The fibrous changes are first seen in those areas showing advanced degeneration and in the region of the portal canals. The capsule of the liver may be very much thickened. In those cases showing advanced fibrosis, extensive proliferations of bile ducts were noted. (Fig. 5.) In two cases examined there was complete cirrhosis of considerable portions of the liver. (Fig. 6.) Large cal-

cified centers were noted in those areas showing the more advanced cirrhotic changes. Few of the livers examined histologically showed an excessive quantity of bile pigment.

The associated hepatic lymph glands exhibited very unusual and rather extensive degeneration of the gland cells. Few of the normal germinal centers could be recognized. A very large proportion of the gland cells had a peculiar, swollen appearance, and in a few places there were fusions of these cells and collections of nuclei simulating

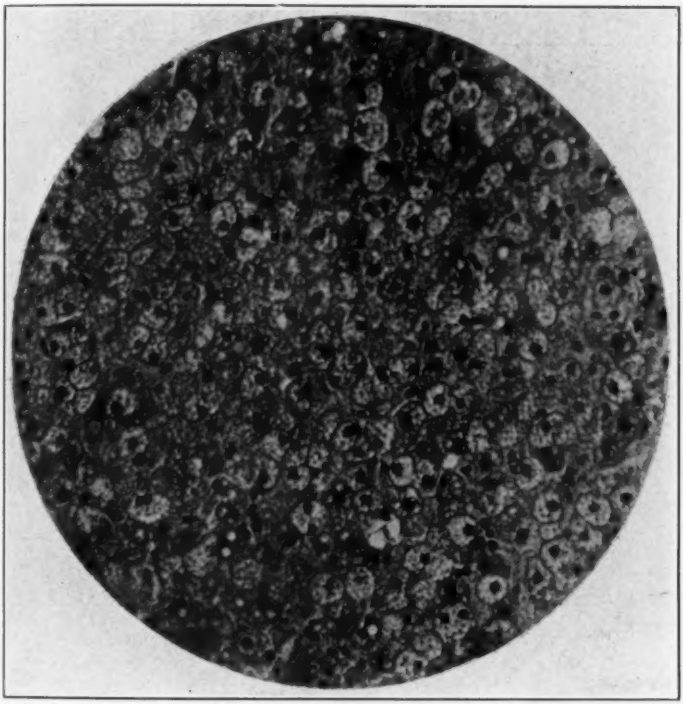


FIGURE 4.—Photomicrograph of section of liver of steer No. 4, showing advanced degeneration of the cells. Practically no normal cells were seen in this specimen. × 620

giant-cell formation. Toward the central portion of the affected glands large areas of cells had been transformed into necrotic, homogeneous masses. (Fig. 7.)

The yellow coloring matter in the livers and lymph glands had evidently been largely removed by the alcohols in the preparation of the tissues for sectioning. Slight traces of the carotene deposits were noted in some of the degenerated and fatty cells of the hepatic lymph glands, but none of the coloring matter could be seen in the liver sections.

RAT-FEEDING EXPERIMENTS

Being unable to understand this rather peculiar proclivity of the bovine liver to store up an excessive amount of carotene while other tissues in the body remained unaffected, the writers decided to conduct some experimental rat feeding with typical yellow livers in order to determine whether these animals would show the same tendency to carotenosis of the liver.

In addition to the liver ration all of the experimental rats received daily a small quantity of corn meal and crushed oats mixed.

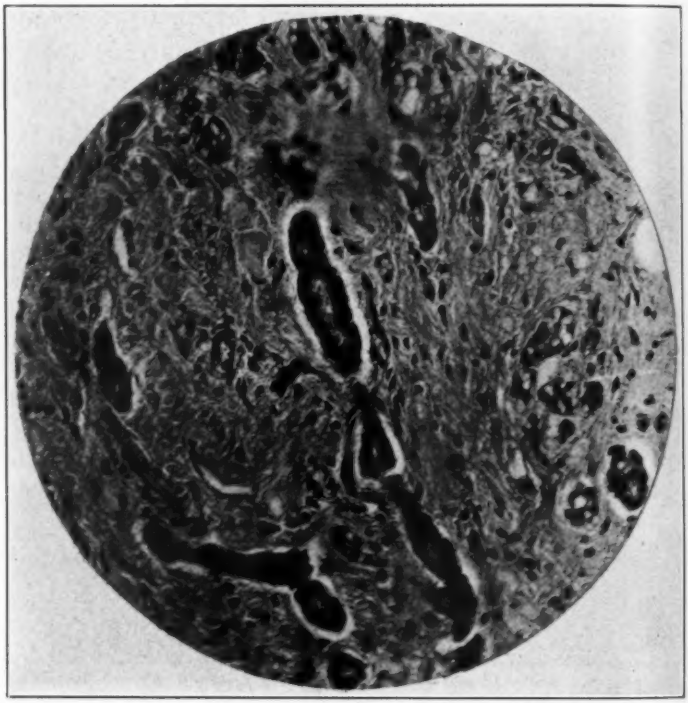


FIGURE 5.—Photomicrograph of section of liver of steer No. 6, showing advanced fibrous changes with extensive bile-duct proliferation. × 620

As a preliminary experiment only three rats were fed. These were mature white rats and received 10 gm. daily of typical yellow liver from steer No. 2 during a period of approximately five weeks.

On post-mortem examination the livers of all the rats in this group were found to be very pale or light in color, but there was no distinct yellowish coloration of the liver tissue. Each liver, however, showed a number of small, whitish areas, evidently necrotic foci. On chemical analysis the three livers failed to show an excessive quantity of carotene, as may be observed in Table 1.

Histologically the livers showed an extensive degeneration of the liver cells. These degenerative changes were more in evidence immediately surrounding many of the central veins and larger blood vessels. (Fig. 8.) In places the complete degeneration and disintegration of the cells had resulted in small, necrotic centers which were seen in sections from all three of the livers. Groups of round cells were observed within and around some of these necrotic centers. (Fig. 9.)

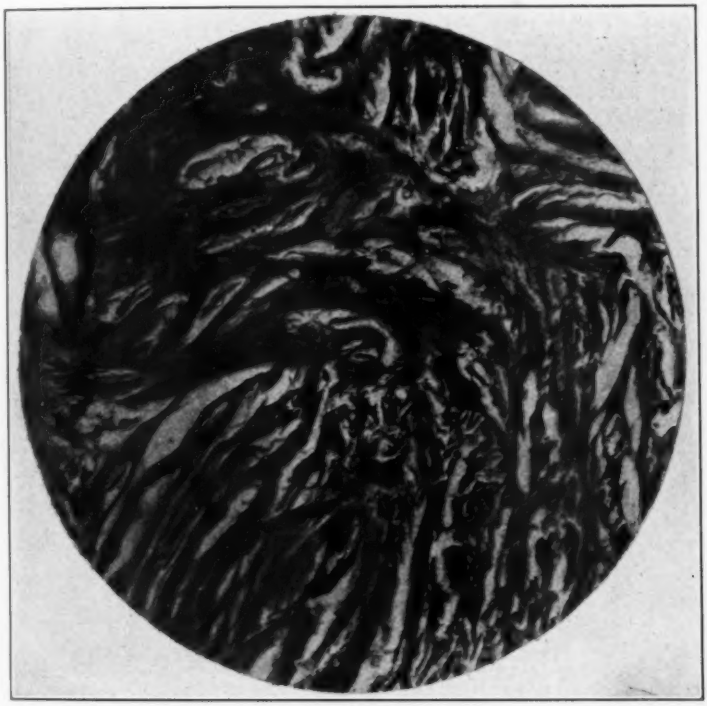


FIGURE 6.—Photomicrograph of section of liver of steer No. 5, showing the complete fibrosis which involved a large portion of the specimen. × 620

While the preliminary feeding experiment gave negative results for carotene deposits, the very interesting findings with respect to the pathological changes in the livers of this small group of rats made it desirable to repeat the experiment in order to ascertain whether this same condition could be produced in a second or third lot of rats. Accordingly arrangements were made to feed two more lots of rats with yellow livers, and at the same time to feed a group of rats a similar quantity of normal bovine liver daily as controls in the experiment.

The rats used in the second feeding experiment were obtained from the Biochemic Division of the Bureau of Animal Industry, and more was known of their previous history and rationing than had been known in the case of the small group used in the preliminary experiment.

Ten rats were used in the second experiment. They were divided into two groups of three animals each, which were fed the yellow livers, and a third group of four rats, which were fed normal bovine

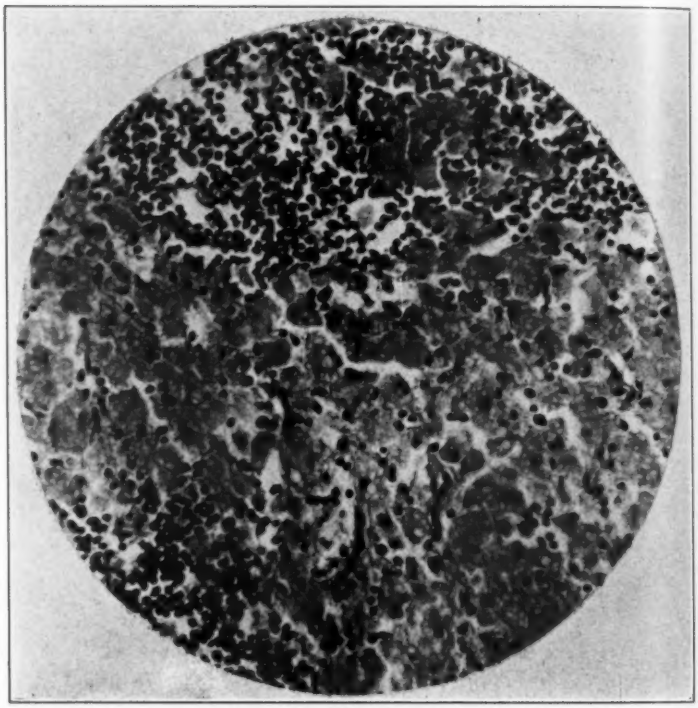


FIGURE 7.—Photomicrograph of section of hepatic lymph gland of steer No. 5, showing extensive areas of degeneration of the cells. × 620

liver. These rats were quite young at the beginning of the experiment, averaging in weight about 40 gm. each.

Lots 1 and 2 were fed liver specimens from steers Nos. 4 and 5, respectively, which were typical cases of carotenosis. Since the rats were rather small they were fed only 5 gm. daily of the liver at first, but during the last half of the feeding period the quantity was increased to 10 gm. daily. Like quantities of normal bovine liver were fed to the four control rats for the same period.

The rats in lots 1 and 2, fed the carotene livers, averaged only 141 gm. in weight at the end of the feeding experiment, an increase of

101 gm., while the rats fed normal liver averaged nearly 154 gm. each, an increase of almost 114 gm.

The post-mortem findings in the livers of rats in lots 1 and 2 were substantially the same as those in the livers of the three rats used in the preliminary feeding experiment; that is, they consisted chiefly of extreme paleness and evidence of necrosis as indicated by numerous small, whitish areas scattered through the livers. The lesions were

FIGURE 8.—Photomicrograph of section of liver of rat No. 3 (first experiment), showing numerous groups of degenerated cells. × 120

somewhat more pronounced in the two lots of the second feeding than in the group of three animals in the first feeding.

The livers of all the rats in lots 1 and 2 were similarly affected, the only difference being the degree of involvement in the different animals. All other tissues of the rats in these two groups appeared to be normal.

The livers of all four of the rats in lot 3, fed normal bovine liver, were found on post-mortem examination to be normal in appearance, and the histological examination of these livers also showed them to be practically normal in structure and appearance. (Fig. 10.)

Histologically the livers of the rats in the two groups fed on carotene livers 4 and 5, in the second feeding experiment, showed more advanced degeneration and necrosis than the livers of the three rats in the preliminary feeding experiment. (Figs. 11 and 12.) The degenerative changes were found to be more extensive and the necrotic foci more numerous. In fact, in some of the specimens there was very little remaining normal liver structure.

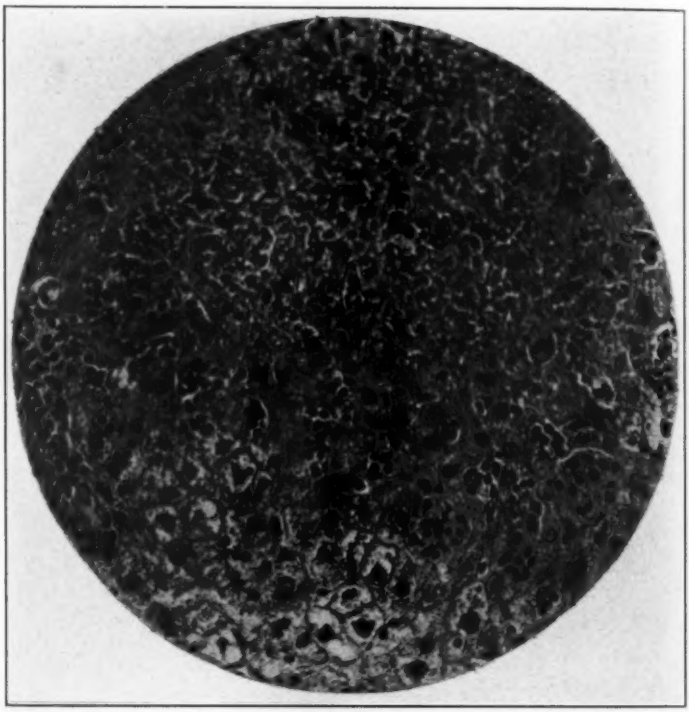


FIGURE 9.—Photomicrograph of section of liver of rat No. 1 (first experiment), showing degeneration of the cells and necrotic center with round-cell infiltration. × 620

There was no decided yellowish coloration of any of the livers in either of the two groups of rats fed on yellow livers in the second experiment, and a chemical analysis of these livers did not show the presence of excessive quantities of carotene in any of the livers of the experimental rats.

ETIOLOGY

The pathological changes found in the different specimens of bovine liver showing carotenosis indicate that the destructive processes in these cases are the result of some form of acute irritation. However, the studies made thus far, which include bacteriological, histological,

and chemical examinations, have failed to reveal the nature of the causative agent.

That the liver changes are due to some specific, toxic substance and that this substance is stored up in the bovine liver tissue in appreciable quantities is shown by the fact that similar destructive changes can be induced in the livers of rats which have been fed for a time on affected cattle livers. The similarity of the degenerative changes in the various cases examined also indicates that the causative element is in all probability of a specific nature.

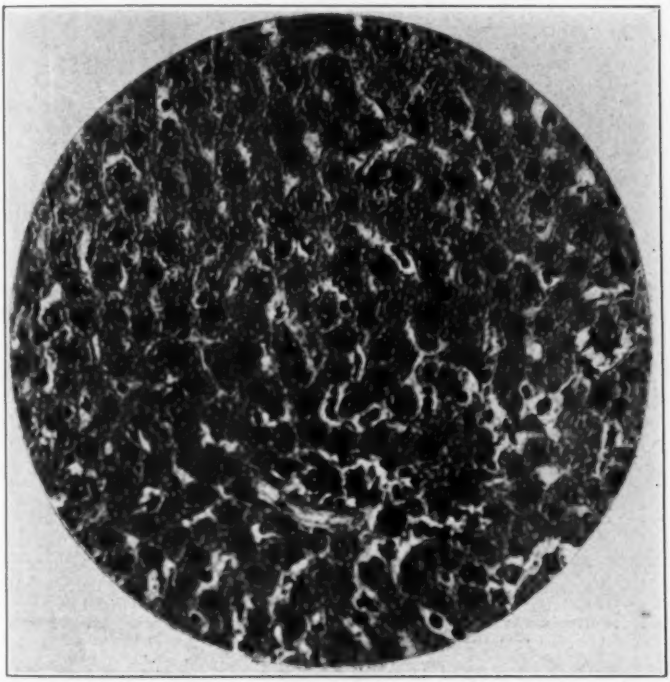


FIGURE 10.—Photomicrograph of section of liver of rat No. 7, control (second experiment). The liver of this rat and those of the other three controls were found to be practically normal. × 620

The fact that the lesions are confined to the liver in cattle and also in the experimental rats suggests that the causative toxic substance has a peculiar affinity for the liver cells; at least it would appear from these observations that for some unknown reason the toxic substance does not pass beyond the liver after reaching that organ.

SUMMARY AND CONCLUSIONS

The results thus far obtained in studies of bovine livers showing carotenosis indicate that the destructive changes found in these livers are, in all probability, due to the presence of a toxic substance, the

nature of which has not yet been determined. That this substance is of a specific nature is indicated by the similarity of the lesions in the different cases studied.

In view of the evidence at hand and the limited available history of the cattle affected, the writers are of the opinion that the causative

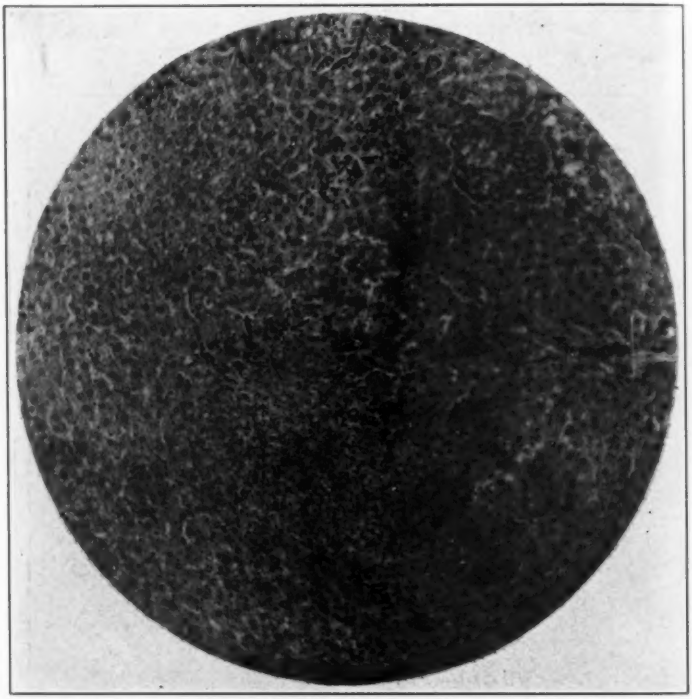


FIGURE 11.—Photomicrograph of section of liver of rat No. 3 (second experiment), showing areas of degenerated cells and one large center of necrosis. × 120

agent, or toxic substance, is a constituent of some plant indigenous to the region or locality where the affected cattle had their origin.

The yellow coloring matter, or pigment, found in the affected livers has been definitely identified as carotene, and it is thought that the carotenosis in these cases is simply an associated condition in which, for some reason difficult to explain, the excess carotene is stored up in the liver, while other tissues of the body remain unaffected.

For the purpose of securing further information relative to this peculiar tendency of the bovine liver to store up carotene in large quantities, feeding experiments were carried out with rats. The rats were fed 5 or 10 gm. daily of typical yellow liver for some weeks. On post-mortem examination the livers of these rats were found to be very pale or light in color, though there was no distinct evidence

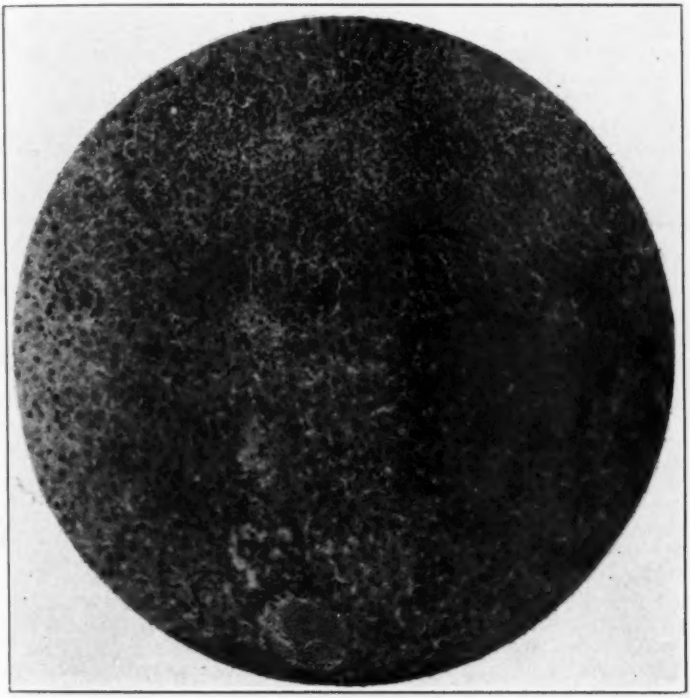


FIGURE 12.—Photomicrograph of section of liver of rat No. 1 (second experiment), showing areas of necrosis with round-cell infiltration. × 120

of yellowing; each of the livers also showed a number of small, whitish areas, which were subsequently found to be necrotic foci. On chemical analysis, however, none of the livers of the experimental rats showed an abnormal quantity of carotene. Control animals fed on normal bovine liver grew more rapidly than animals fed yellow livers of high carotene content.

THE INSECTICIDAL ACTION OF SOME DERIVATIVES OF PYRIDINE AND PYRROLIDINE AND OF SOME ALIPHATIC AMINES[1]

By C. H. RICHARDSON, formerly *Entomologist*,[2] and H. H. SHEPARD, *Assistant Entomologist, Division of Deciduous Fruit Insects, Bureau of Entomology, United States Department of Agriculture*[3]

NATURE OF THE STUDY

The investigation reported in this paper is a study of the relative toxicity of various nitrogenous organic compounds when used as contact insecticides. These compounds were synthesized by F. B. La Forge, of the Bureau of Chemistry and Soils, and submitted by him to the Bureau of Entomology for a study of their insecticidal value.[4] Many of the compounds are related more or less closely to nicotine or to those simpler substances, pyridine and methylpyrrolidine, which are contained in the nicotine molecule. A few compounds are of insecticidal interest mainly for other structural characteristics of the molecule or as substances intermediate in the synthesis of some of the compounds.

This investigation has two objects: (1) To find some compound having a toxicity comparable to that of nicotine which can be synthesized for practical use; and (2) to make some contribution toward the explanation of the unique toxicity of nicotine. A considerable number of compounds have been investigated, very few of which show much toxicity, and none have the extreme toxicity of nicotine. Some of the more toxic compounds will be tested further.

EXPERIMENTAL METHODS

Essentially the same method has been used in the determination of toxicity as was employed by Richardson and Smith (*10*). Nasturtium plants bearing colonies of *Aphis rumicis* L. were set in bottles of water, the stems passing through perforated rubber stoppers. The sprays used were applied at a constant pressure of 5 pounds per square inch by an atomizer connected to an air-pressure line into which a pressure regulator and gauge were inserted. After the applications were made each plant was set aside on a paper bordered with a sticky tree-banding material to prevent the escape of the aphids. After any excess spray had drained down the stem a collar of cardboard was placed around each plant just above the stopper to prevent any of the aphids from falling into the accumulation of spray material at the base of the stem. After from 20 to 24 hours the percentage of aphids killed and the condition of the plants were determined. The figures in this paper are based on counts in which all sizes of aphids are included. Separate record has been made of the adult aphids killed in

[1] Received for publication Nov. 12, 1929; issued May, 1930.
[2] Resigned June 20, 1928.
[3] The writers are indebted to R. C. Burdette for assistance in some of the experiments.
[4] This is part of a cooperative project between the Insecticide Division, Bureau of Chemistry and Soils, and the Division of Deciduous Fruit Insects, Bureau of Entomology, the chemical part of which has already been published (*4*, *5*, *6*).
[5] Reference is made by number (italic) to Literature Cited, p. 1014.

the more recent tests. In the period from May, 1927, through June, 1928, over 65,000 aphids were counted, of which 11.7 per cent were adults.

Most of the compounds tested are miscible in water. A few had to be dissolved in a little acetone or other solvent and then shaken up in the spreader to emulsify them.

COMPARISON OF SOAP AND SAPONIN AS SPREADERS IN THE EXPERIMENTS

Richardson and Smith (*10, p. 8*) in 1923 reported that a spray of 0.3 per cent sodium fish-oil soap killed an average of 14 per cent of a population of *Aphis rumicis*. Since the date of these earlier experiments further tests of soap as a toxic agent have been made which tend to show that the earlier figure of toxicity was considerably too low and to suggest the use of some less toxic spreader for the tests. These later tests covered a period of about a year and showed a mean kill of 46.9 per cent in a total population of nearly 6,500 aphids used as controls. Still more recently a test of 0.3 per cent soap was made on a colony of about 400 aphids in which 44 per cent of all sizes and 28.8 per cent of the adults were killed.

Tattersfield, Gimingham, and Morris (*14, p. 64; 15*) used a 1 per cent saponin solution as a wetting agent in their tests of insecticidal action. In view of a considerable reduction in the toxicity due to the spreader when 1 per cent saponin is used, this material has been utilized in the later tests in the present investigation. Controls, in which over 2,500 aphids have been employed at various dates since 1925, showed when sprayed with 1 per cent saponin an average kill of 14.4 per cent of the aphids. There are other points relative to wetting and emulsification in which either soap or saponin excel. The relative toxicity of the wetting agent is, however, the character of the most importance here, and the other qualities will not be dealt with at this time.

BASIS OF COMPARISON OF EFFECTIVENESS OF SPRAYS

As in previous tests (*10, p. 2*) the toxic concentration has been "considered to be the minimum capable of killing about 95 per cent of the aphids." As the concentration which kills 50 per cent of the aphid population is a more valuable point, statistically, for comparative purposes, both this "median dose" to kill 50 per cent and the "practical dose" to kill 95 per cent have been tabulated.

The criterion of tolerance of the plant to a compound was the same as that described in the paper just cited (*10, p. 2*).

RESULTS OF THE EXPERIMENTS

The data here presented have been tabulated from curves which were made from all the observations, and the figures given in Tables 1 and 2 show the approximate (or actual) concentrations at which the mortality amounted to 50 or 95 per cent. Table 1 shows the results thus obtained when the spray carried 0.3 per cent of sodium fish-oil soap as a spreader and Table 2 the results obtained later with 1 per cent of saponin. The same compounds were not always available when the two series of tests were being made.

TABLE 1.—*Toxicity of various compounds based on the concentration necessary to kill 50 and 95 per cent of Aphis rumicis on nasturtium leaves, and the plant tolerance shown, with 0.3 per cent of sodium fish-oil soap used as a spreader*

Compound	Experiments	Range of concentrations tested	Toxic concentration necessary to kill approximately—		Tolerance of plant
			50 per cent	95 per cent	
	Number	*Grams per 100 c. c.*	*Grams per 100 c. c.*	*Grams per 100 c. c.*	*Grams per 100 c. c.*
Nicotine and closely related compounds:					
Nicotine	10	0.00025–0.05	[a] (0.0003)	(0.007)	>0.05
Nicotyrine	4	.005 – .1	(.004)	.05	>.1
Metanicotine	5	.005 – .44	(.003)	.05	>.44
Dihydrometanicotine	5	.01 – .5	(.03)	.5	.5
Methylmetanicotine	3	.01 – .1	<.01	.1	>.1
Benzoylmetanicotine	4	.01 – .2	.01	>.2	>.2
Pyridine and derivatives:					
Pyridine				25	25 –30
Pyridine vinyl bromide	3	.5 –2.0	2.0	>2.0	>2.0
Pyridine allyl bromide	7	.05 –5.0	(.03)	.25	.25– .5
Dipyridine ethylene ammonium chloride	3	.25 –1.0	(.15)	1.0	1.0
β-pyridylcyanide	5	.25 –3.0	.5	>3.0	>3.0
β-pyridylethylamine	5	.05 –1.5	.3	1.5	<.5
β-pyridylethyl-N-ethylamine	4	.01 – .5	.03	>.5	<.5
β-pyridyl-n-butylamine	4	.05 –1.0	.1	>1.0	<1.0
β-pyridyl-n-butyl-N-methylamine	2	.5 –1.0	<.5	>1.0	<.5
Pyrrole, pyrrolidine and derivatives:					
Pyrrole	7	2.5 –18.0	5.0	18.0	2.5
Pyrroline hydrochloride	3	.1 – 1.0	.1	(.8)	>1.0
Pyrrolidine hydrochloride	6	.1 – 1.2	.1	1.0	.7
N-methylpyrrolidine hydrochloride	3	.1 – 1.0	(.25)	1.0	1.0
N-ethylpyrrolidine hydrochloride	5	.1 – 1.0	.25	1.0	.8
N-n-butylpyrrolidine hydrochloride	4	.1 – 1.0	(.2)	.5	.5
N-benzylpyrrolidine	4	.1 – 1.0	(.08)	(.4)	.25– .5
α-methylpyrrolidine hydrochloride	5	.065 – 1.0	(.12)	.5	.5
α-methyl-N-benzylpyrrolidine	4	.05 – .5	.1	.5	>.5
α-phenyl-N-methylpyrroline	5	.05 – 2.0	(.8)	2.0	.25– .5
α-phenyl-N-methylpyrrolidine	6	.1 – 1.0	(.15)	(.6)	.5(?)
α-phenylpyrrolidine	3	.25 – 1.0	<.25	.5	(?)
α-α-dimethylpyrrolidine oxalate (neutral)	3	.1 – 1.0	(.08)	.5	.1
Aliphatic amines and derivatives:					
Vinyltrimethylammonium hydroxide (neurine)	3	.25 – 1.0	(.2)	.5	0.25
Allyltrimethylammonium hydroxide	5	.05 – 2.0	(.3)	1.0	<.5
Allyltrimethylammonium bromide	3	.5 – 2.0	<.5	(.8)	.5
Phenyl-n-butyl-N-methylamine	4	.25 – 2.0	.5	(1.5)	.25
Phenylallyl-N-methylmethylamine[b]	4	.25 – 2.0	.5	(1.5)	.25– .5

[a] Figures in parentheses are estimated from points on the toxicity curves. The other figures are from readings very close to or directly at the points where the mortality was 50 or 95 per cent.

[b] The compound designated by this name has 3 possible formulas (4); i. e., $C_6H_5CH_2(NHCH_3)CH_2CH{=}CH_2$, $C_6H_5CH{=}CHCH_2CH_2NHCH_3$, or $C_6H_5CH{=}CHCH(NHCH_3)CH_3$. The first one is phenylallyl-N methylmethylamine.

TABLE 2.—*Toxicity of various compounds based on the concentration necessary to kill 50 and 95 per cent of Aphis rumicis on nasturtium leaves, and the plant tolerance shown, with 1 per cent of saponin used as a spreader*

Compound	Experiments	Range of concentrations tested	Toxic concentration necessary to kill approximately		Tolerance of plant
			50 per cent	95 per cent	
Nicotine and closely related compounds:	*Number*	*Grams per 100 c. c.*	*Grams per 100 c. c.*	*Grams per 100 c. c.*	*Grams per 100 c. c.*
Nicotine	8	0.002–0.2	(0.015)	(0.08)	>0.2
Metanicotine	3	.02 – .5	(.04)	(.4)	>.5
Methylmetanicotine	3	.02 – .5	(.02)	(.3)	>.5
Propylmetanicotine	3	.02 – .5	.1	(.4)	.1
Pyridine derivatives:					
Pyridine vinyl bromide	2	.5 –1.0	(1.5–2.0)		>1.0
Pyridine allyl bromide	3	.02 – .5	.1	>.5	>.5
β-2-allylpyridine	3	.02 – .5	>.5		>.5
β-Pyridylphenylmethylamine	3	.02 – .5	>.5		>.5
Dimethyl-β-pyridyl carbinol	3	.02 – .5	>.5		>.5
Benzylpyridine, (b. p. 260°–280° C.)	6	.005– .5	(.04)	.5	.2
Benzylpyridine (b. p. 280°–300° C.)	4	.02 – .5	.02	(.4)	.2
Benzylpyridine (b. p. 300° C. and over)	3	.02 – .5	(.05)	.5	.1
α-benzylpyridine	4	.005– .5	.02	.5	.1–.5
β-benzylpyridine	3	.02 – .5	.5	>.5	.1
γ-benzylpyridine	3	.02 – .5	.1	>.5	>.5
Benzylpiperidine (b. p. 260°–270° C.)	3	.02 – .5	(.2)	>.5	>.5
α-dihydrostilbazol	3	.02 – .5	.02	.5	.1
Pyrrolidine derivatives:					
Pyrrolidine hydrochloride	3	.1 –1.0	(.7)	>1.0	(?)
α-methylpyrrolidine hydrochloride	3	.125– .5	(.4)	>.5	>.5

The most toxic compounds that have been tested in this study are nicotine and the closely related compounds, nicotyrine and metanicotine, which were synthesized from it. Nicotine is the only one of these three compounds that occurs in any great quantity in nature. The figures in Tables 1 and 2 show nicotyrine and metanicotine to be very close to each other in relative toxicity, nicotine being from 3 to 13 times as toxic, according to the spreader used in the test spray and whether the relative toxicity is based on the concentration needed to kill 95 or 50 per cent of the aphids. As a measure of this closeness one may compare the magnitude of other differences in toxicity; i. e., nicotine is about 100 times as toxic as dihydrometanicotine, 150 times as toxic as N-methylpyrrolidine hydrochloride and 3,500 times as toxic as pyridine. A few of the most toxic compounds are being examined by more exact methods than those employed in the tests described in this paper.

There are very few references of a quantitative nature to the pharmacological action of metanicotine and nicotyrine. Fränkel (*2, p. 426*), quoting Falck and Ringhardtz (*11*), says metanicotine and nicotine act in the same manner, but a dose of metanicotine nine times a given dose of nicotine required twice as long in which to kill.

The toxicity figures for pyridine are taken for purposes of comparison from a previous paper (*10, p. 4*) where a discussion of the literature on the toxicity of that compound may be found. Pyrrole apparently has a toxicity of the same order as pyridine, whereas all the pyrroline and pyrrolidine compounds investigated are very much more toxic. The pyridine compounds studied, with the exception of the benzylpyridines and dihydrostilbazol, which will be discussed later, are in general less toxic than the pyrroline and pyrrolidine compounds.

TOXICITY AND CHEMICAL STRUCTURE

Richardson and Smith (*10, p. 11*) discussed the effect of hydrogenation of the cyclic nucleus of some compounds, to increase toxicity in the case of pyridine and piperidine, and to decrease it in the case of nicotine and hexahydronicotine. In the present investigation it has been found that the partial hydrogenation of pyrrole to pyrroline increases toxicity about as much as complete hydrogenation (or reduction) to pyrrolidine. Hydrogenation of α-phenyl-N-methyl pyrroline to the corresponding pyrrolidine, however, makes it from 4 to 8 times as toxic. The increase in toxicity of pyrrolidine over pyrrole is considerably greater than in the change of pyridine to hexahydropyridine (piperidine). Nicotyrine (β-pyridyl-α-N-methylpyrrole) is from 7 to 10 times less toxic than nicotine (β-pyridyl-α-N-methylpyrrolidine). This increase in toxicity on the addition of hydrogen to unsaturated N-heterocyclic compounds is attributed by Fränkel (*2, p. 26*) to an increase in reactivity of the nitrogen when it passes from the tertiary to the secondary grouping in the reduction of pyridine. Oswald (*7, p. 326*) follows the preceding writer in saying that hydrogenation increases the activity of the nitrogen in pyridine. He adds, however, the statement that, in the case of pyrrole in which the nitrogen is secondary instead of tertiary as in pyridine, no considerable increase is discernible in its reduction to pyrrolidine, a point which is not upheld by the present results.

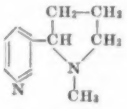
Oswald (*7, p. 328*) further reports that unsaturated side chains increase toxicity more than do saturated ones. This is difficult to harmonize with the decrease in toxicity which seems to be rather general in the case of unsaturated rings. However, the fact that the mere addition of two hydrogen atoms to satisfy a double bond in the side chain of metanicotine reduces the toxicity 100 times is in agreement with Oswald's statement.

Fränkel (*2, p. 66*) states that methylation of the nitrogen reduces toxic action. Oswald (*7, p. 328*), on the other hand, says that N-methylaniline and N-methylpiperidine are more active, respectively, than aniline and piperidine. Browning et al. (*1, p. 337*) report that substitution of methyl and ethyl radicles into NH_2 groups of diaminoacridine lessens the toxicity. Several instances of methylation may be cited among the compounds tested in the present investigation, but none show any effect upon toxicity that may be considered significant. Methyl-, ethyl-, and normal butyl-pyrrolidine hydrochlorides have about the same toxicity as the simple pyrrolidine hydrochloride. (Table 1.) β-pyridyl-n-butyl-N-methylamine has practically the same toxicity as β-pyridyl-n-butylamine. (Table 1.) The same is true in the case of α-phenyl-N-methylpyrrolidine and α-phenylpyrrolidine (Table 1) and in the case of metanicotine, methylmetanicotine, and propylmetanicotine (Table 2).

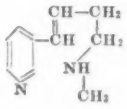
Methylation of the alpha carbon atom in N-benzylpyrrolidine produces a small but definite reduction in toxicity.

The cyclic pyrrolidine ring of nicotine may be replaced by an aliphatic amine group, as in metanicotine, with the destruction of only a part of the original toxicity of the former compound. It is therefore possible that other aliphatic amines of simpler structure might be substituted in the β position of pyridine to form compounds that would be highly toxic to insects.

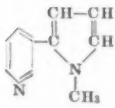
A few of the compounds tested, the structural formulas of which are similar to that for nicotine, are represented in the descending order of their toxicity as follows:



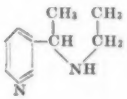
(1) Nicotine



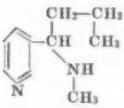
(2) Metanicotine



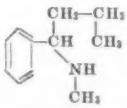
(3) Nicotyrine



(4) β-Pyridylethylethylamine



(5) β-Pyridyl-n-butyl-N-methylamine



(6) β-Phenyl-n-butyl-N-methylamine

Oswald (*8, p. 430, 436*), referring to the pharmacological action qualitatively, says that α-α-pyridylpyrrole acts on frogs similarly to β-pyridyl-N-pyrrole and to β-pyridyl-α-N-methylpyrrole (nicotyrine). He also reports that nicoteine (β-pyridyl-α-N-methylpyrroline) is much more toxic than nicotine.

Tattersfield (*12*) thinks "the whole make-up of the nicotine molecule, including its spatial arrangement, contributes to its toxic properties." Tattersfield and Gimingham (*13, p. 235*) state that "nicotine undoubtedly owes its high potency to its molecular make-up taken as a whole; the attachment of the pyrrolidine nucleus in the β-position of the pyridine ring, and the presence of an asymmetric carbon atom may be significant."

TOXICITY OF BENZYLPYRIDINE

Preliminary tests of benzylpyridine showed that it held some promise as an effective insecticide that could be readily synthesized (La Forge (*6*), also Tattersfield and Gimingham (*13, p. 236*)). As these tests were continued it became apparent that a large share of the toxicity might be due simply to the immiscibility in water (oily nature) of benzylpyridine, hence the comparative tests reported in Table 3 were made.

TABLE 3.—*Comparison of effects of benzylpyridine (b. p. 270°–310° C.) and of a naphthenic-base petroleum oil (viscosity 105 seconds at 100° F.) sprayed on Aphis rumicis on nasturtium leaves*

Spray composition	0.5 per cent spray		0.1 per cent spray		0.02 per cent spray	
	Total aphids in test	Mortality	Total aphids in test	Mortality	Total aphids in test	Mortality
	Number	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*
Naphthenic base petroleum oil:						
Emulsified with 1 per cent saponin	314	56.4	628	43.2	(?)	• 15
Emulsified with fish-oil soap (cold-mix formula)	824	91.3	1,027	51.1		
Benzylpyridine:						
Emulsified with 1 per cent saponin	• 300	98+	334	74.8		
Emulsified with fish-oil soap (cold-mix formula)	733	98.5	1,306	71.1	1,261	31.7
Acid solution (1 per cent saponin as spreader)	328	78.6	403	52.6		
Acid solution emulsified with ammonia (1 per cent saponin as spreader)	• 300	97+	245	75.9		
Acid solution (0.3 per cent fish-oil soap as spreader)	• 300	99+	369	89.2		

• Estimated.

All the preliminary tests were made with spray solutions in which the required quantity of benzylpyridine was emulsified by shaking with a 10 per cent saponin solution and was then diluted to the required strength. These solutions had to be shaken frequently and used within an hour or two so that the oil would not separate out at the bottom of the flask. In all cases the 0.5 per cent solutions were milky emulsions, whereas the 0.1 and 0.02 per cent solutions were fairly clear.

It was found that the 1 per cent saponin emulsions were not suitable for properly comparing a petroleum oil with benzylpyridine. The naphthenic oil separated out in much larger oil globules than did the benzylpyridine at the same concentration. Hence a cold-mix soap emulsion of each material was made according to the formula of Richardson and Griffin (*9*), except that sodium fish-oil soap instead of potassium fish-oil soap was used. Table 3 shows that benzylpyridine has about the same effect in either type of emulsion notwithstanding the much greater toxicity of the soap. In the case of the naphthenic oil the soap emulsion still gives a toxicity somewhat below that of benzylpyridine but much higher than that of the imperfect saponin emulsion. The droplets of oil in the latter emulsion were larger and toxicity should have been higher (*3*), but because the oil droplets were so variable in size and so unevenly distributed in the solution, it was impossible to select samples which had the same oil concentration.

At the suggestion of Doctor La Forge benzylpyridine was dissolved in dilute hydrochloric acid and again thrown out of solution by neutralizing with dilute ammonia water, the benzylpyridine appearing in the form of extremely small droplets that remain suspended for several days.

Spray tests were made with these solutions and emulsions. It was found that a solution of benzylpyridine titrated with hydrochloric acid in the presence of methyl red in which there could be no complication owing to the presence of large oily droplets of benzylpyridine showed a greatly reduced toxic action, whereas the toxicity imme-

diately appeared again upon the addition of ammonia water, thus releasing the benzylpyridine in the form of tiny oil droplets.

SUMMARY

Numerous nitrogenous organic compounds have been tested as contact insecticides against *Aphis rumicis* L. colonized on nasturtium plants. These compounds for the most part are structurally related to nicotine.

Metanicotine and nicotyrine are the only substances tested which show a toxicity approaching that of nicotine.

The relation of chemical structure to toxicity, particularly hydrogenation and methylation of certain groups, is briefly discussed.

Benzylpyridine appears to have some promise as an insecticide for special uses where its oily properties are advantageous, and yet where an ordinary cheap oil is not suitable.

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DEVELOPMENT OF COTTON FIBERS IN THE PIMA AND ACALA VARIETIES[1]

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INTRODUCTION

The spinning value of cotton fibers is dependent in large measure on their length and degree of maturity. The thickness of fiber walls is closely correlated with degree of maturity and fiber strength. Other fiber characters influence the spinning quality of cotton, but those mentioned are of major importance and are the ones which were studied in this particular investigation.

The extent to which factors external to the cotton plant, such as soil and climatic conditions, influence fiber length and maturity is not well understood. A knowledge of the life history of cotton fibers from the flowering of the plants, at which time fiber growth begins, to the maturity of the fibers is necessary in order to establish the degree to which external factors influence the length of fibers and the thickness of the fiber walls. The only published information relative to the development of cotton fibers which has come to the author's attention is the work of Balls [2] in Egypt. His material was taken from a single series in which the flowers opened near the beginning of the flowering period. The variety used was a pure strain of Egyptian No. 77 which has a fiber length of about 30 mm. as determined by measurements taken on combed fibers attached to the seed. Balls found that the fibers increased in length until the twenty-fifth day after flowering. The maximum rate of growth in length occured near the fifteenth day after flowering. Thickening of the fiber walls began about 21 days after flowering, reached its maximum rate of increase in 36 to 39 days, and was practically completed 45 days after flowering.

The data included in the present publication relate to the life history of two types of cotton fibers developing in successive periods during the growing season of 1926.

MATERIAL AND METHODS

Pima, the only variety of American-Egyptian cotton now grown in the Southwest, has a fiber length of approximately 40 mm. (1 9/16 to 1 5/8 inches). Acala cotton is the upland variety most widely grown in the Southwest, and has a fiber length of 28 to 30 mm. (1 1/8 inches). These two varieties were chosen for the work on fiber development because they are representative of the two types of cotton grown commercially in the Southwest.

The fiber material studied was obtained from Acala and Pima plants growing on adjacent plots at the Salt River Valley Experiment Station. The plants were irrigated with sufficient frequency to prevent

[1] Received for publication Aug. 6, 1929; issued May, 1930.

[2] BALLS, W. L. THE DEVELOPMENT AND PROPERTIES OF RAW COTTON, p. 73-85, illus. London, 1915.

abnormal water stress but not so frequently as to produce overgrown plants. The soil in these plots is a fertile clay loam with a moisture equivalent of approximately 19.

Several hundred flowers of each variety were tagged on July 13, August 3, August 24, and September 14, making four series at 3-week intervals. A study of Figure 1 shows that the July 13 series began near the fore part of the flowering period and the September 14 series near the close of the flowering period. The August 3 and August 24 series flowered during mid season when flowering was at its height. Six bolls were collected from each series at 3-day intervals from flowering to maturity and stored in 5 per cent formalin for later study.

DEVELOPMENT OF FIBER LENGTH

Three measurements were made of the length of the wet fibers in each boll immediately after the bolls were taken from the preserving

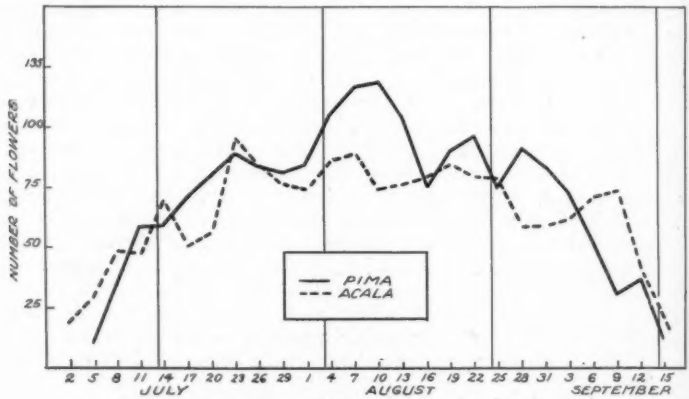

FIGURE 1.—Rate of flowering on 25 plants of the two cotton varieties studied

solution. Thus 18 measurements were made for each 6-boll collection. The fibers in the 3-day-old bolls were too short to measure satisfactorily without the aid of a microscope, but succeeding measurements were made without one.

INCREASE IN FIBER-WALL THICKNESS

Several hundred fibers were taken from each boll and infiltrated and embedded in paraffin in preparation for sectioning. (Celloidin and gelatin are also used for the infiltration of textile fibers with certain advantages over the paraffin method.) The embedded material was sectioned with a microtome having a drawing cut. Considerable difficulty was experienced in cutting the young fibers before the walls began to thicken, but as the walls thickened the fibers were more easily sectioned. The sections were mounted with albumen glycerin and stained with gentian violet.

The rate of wall thickening is relatively slower than the increase in fiber length, and it was soon found that measurements taken on material collected at 6-day intervals furnished dependable information on this development.

RESULTS

Cotton fibers are unicellular elongations from the epidermal layer of the ovule. The fibers begin to extend beyond the epidermis almost immediately after fertilization and are plainly visible under proper magnification 24 hours later. A comparison of Figures 2, 3, and 4 shows the rapidity of fiber growth following fertilization. Some fiber growth occurs even in ovules in which fertilization has been prevented by the removal of the anthers before the flower has opened. Fiber growth in unfertilized ovules is much slower for the first three

FIGURE 2.—Cross section of cotton ovule showing appearance of epidermis on day of fertilization. No transformation of epidermal cells into fibers is evident. Highly magnified

days than in fertilized ovules, and very little if any growth takes place after the third day, abscission of the boll usually occurring within seven or eight days after flowering.

The cumulative increase in fiber length of both Acala and Pima cotton is shown in Figure 5 and in Tables 1 and 2. Increase in length was practically completed in the first three series of Acala fibers in 21 days and in the corresponding series of Pima fibers in 27 days after flowering. The September 14 series of each variety required three days longer for the completion of fiber elongation, or 24 and 30 days for Acala and Pima, respectively. It will be noted that the temperatures were declining rapidly during the time when

the September 14 series of Acala and Pima fibers were developing length. The minimum temperatures had dropped considerably below 60° F., and the maximum temperatures had declined at least 10°. Pima cotton required about six days longer than Acala to complete growth in fiber length due largely to the fact that the Pima fibers were 10 mm. longer than the Acala. Dry fibers from mature bolls were used for the final measurements in each series; wet fibers from immature green bolls were used for all other measurements. This accounts for the drop at the end of each curve.

The curves showing the cumulative increase in fiber length in each series of Pima cotton have been superimposed in Figure 6 and those

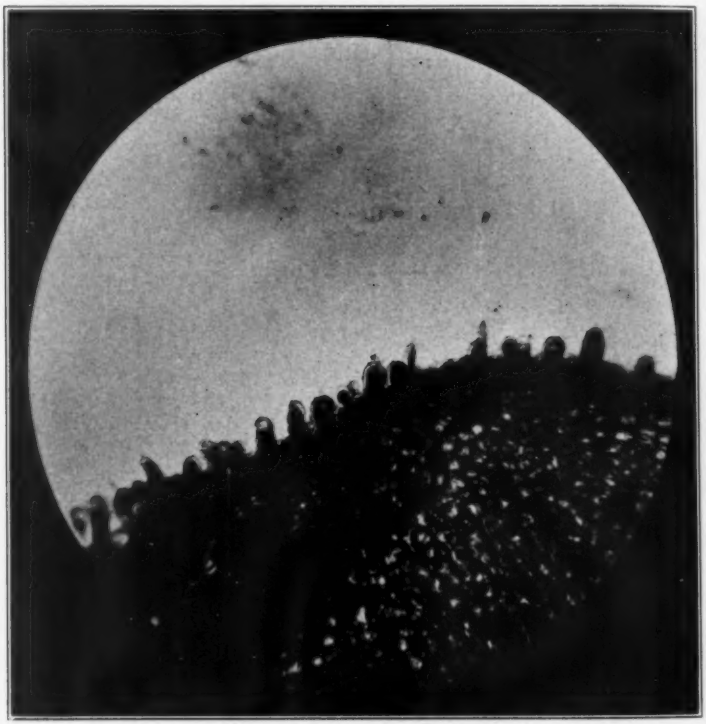

FIGURE 3.—Cross section of cotton ovule showing beginning of fiber growth from epidermal layer 24 hours after fertilization. Highly magnified

of Acala in Figure 7. These figures show that prevailing conditions did not create any great differences between either the Acala or the Pima series in the increase in length.

The rate of increase in fiber length is shown in Figure 8. The rate of fiber elongation was materially greater with Acala fibers than with Pima fibers after the ninth day from the flowering period. The most rapid increase occurred three days earlier with the Acala fibers than with the Pima in the first two series and six days earlier in the last two series. The greatest increase in fiber length was about 8

mm. in three days, the Pima fibers making slightly more rapid growth than the Acala in the first three series and a full millimeter more in the September 14 series. This means that these cotton fibers were making a daily growth in length of from three-thirty-seconds to one-eighth inch at the time of their most rapid growth. The temperatures which prevailed during the development in fiber length did not appear to cause any material differences in the rate of growth in the first three series but prolonged the period of growth in the September 14 series.

The curves showing the rate of fiber elongation have been superimposed in Figures 9 and 10. The most rapid increase in length of

FIGURE 4.—Cross section of cotton ovule showing development of fibers 48 hours after fertilization. Highly magnified

the Pima fibers occurred during the 3-day period ending on the twenty-first day after the flowering period in all series, as shown in Figure 9, while the most rapid increase in length of the Acala fibers occurred during the 3-day period ending on the fifteenth day in the August 24 and September 14 series and during the period preceding the eighteenth day in the July 13 and August 3 series, as shown in Figure 10. Present data do not indicate the factors which caused the retardation in the rate of fiber elongation from about the ninth to the fifteenth day in all of the Pima series and also in the Acala series to a lesser degree from the ninth to the twelfth day.

TABLE 1.—*Development of fiber length in Pima cotton from flowering to maturity*

Age of bolls (days)	Mean length of lint with ±⅝ in—			
	July 13 series	Aug. 3 series	Aug. 24 series	Sept. 14 series
	Mm.	*Mm.*	*Mm.*	*Mm.*
3	0.4±0.041	0.4±0.050	0.3±0.047	0.3±0.028
6	2.9± .543	3.0± .159	2.7± .222	2.4± .960
9	7.1± .667	6.7± .299	6.1± .331	5.8± .455
12	11.9± .597	11.0± .704	10.9± .554	10.0± .450
15	17.0± .959	16.4± .712	15.2± .471	13.3± .654
18	24.4±1.635	23.2± .424	22.8± .361	19.8± .705
21	32.4± .880	31.7±1.043	31.1± .390	28.8± .575
24	39.1± .882	37.5± .915	37.8± .522	35.6±1.204
27	40.5±1.355	39.7± .829	39.2± .876	38.5±1.140
30	40.4± .782	40.1± .527	39.7± .533	39.9± .312
33	40.6± .749	40.3± .657	39.8± .719	39.9± .470
36	40.8± .875	40.1± .524	39.9± .314	39.9± .805
39	41.4± .518	40.9± .566	39.9± .555	40.2± .500
42	40.4± .676	40.2± .601	40.0± .471	40.4± .755
45	40.9±1.053	40.2± .381	40.3± .463	40.0± .666
48	41.0± .499	40.2± .533	40.0± .745	40.1± .424
51	40.7± .650	40.2± .533	40.1± .705	40.1± .458
54	37.5± .590	40.4± .558	40.1± .705	40.3± .443
57		37.9± .741	40.3± .666	40.1± .458
60			39.9± .404	40.1± .404
63			36.9±1.282	40.5± .678
66				40.1± .229
69				40.3± .652
72				40.1± .458
75				40.1± .566
78				40.1± .404
81				40.3± .471
84				38.7±1.609
Average ratio of mean length to standard deviation	40.50	51.73	57.35	63.60

TABLE 2.—*Development of fiber length in Acala cotton from flowering to maturity*

Age of bolls (days)	Mean length of lint with ±⅝ in—			
	July 13 series	Aug. 3 series	Aug. 24 series	Sept. 14 series
	Mm.	*Mm.*	*Mm.*	*Mm.*
3	0.6±0.033	0.7±0.048	0.6±0.065	0.6±0.055
6	3.5± .236	3.2± .818	3.2± .206	3.0± .348
9	7.8± .500	6.5± .579	7.3± .883	7.2± .499
12	13.3± .628	13.2± .858	11.5± .833	12.2± .533
15	20.7± .943	19.5± .490	19.6± .755	20.2± .785
18	28.3± .926	27.9± .432	26.9± .911	25.3± .458
21	30.0± .705	30.1± .681	29.7± .533	28.5± .500
24	30.0± .779	29.8± .552	29.8± .415	30.1± .524
27	29.9± .657	29.9± .755	29.9± .524	30.1± .404
30	29.7± .448	29.9± .547	29.7± .577	29.7± .558
33	30.1± .524	29.7± .496	29.9± .737	29.7± .779
36	30.0± .624	29.9± .585	29.7± .658	29.9± .524
39	30.0± .577	29.9± .514	29.7± .869	30.3± .810
42	30.1± .737	29.9± .657	29.8± .533	29.8± .566
45	30.0± .823	30.0± .666	30.0± .471	19.3± .816
48	26.0±1.328	30.1± .458	29.9± .657	30.2± .415
51		29.9± .566	29.7± .309	30.3± .833
54		27.8±1.771	27.5±1.300	29.9± .810
57				29.8± .975
60				30.0± .745
63				30.1± .524
66				30.1± .565
69				30.2± .500
72				29.8± .785
75				28.4±1.458
Average ratio of mean length to standard deviation	36.53	40.89	39.47	41.86

The standard deviation from the mean fiber length was exceptionally small with both varieties, as indicated in Tables 1 and 2. Deviations were much greater with the young fibers from 3 to 18 days old, probably due to the greater likelihood of error in measurements taken on short fibers as compared with those taken on the longer and older

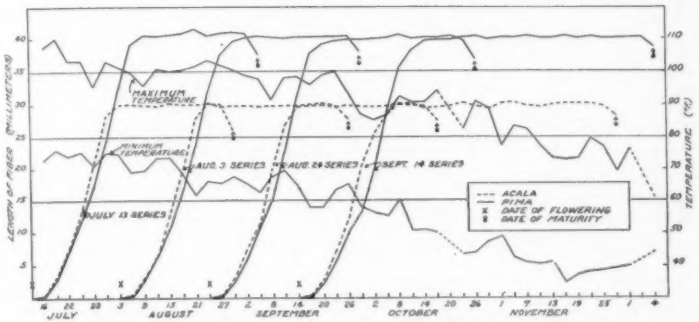

FIGURE 5.—Cumulative increase in fiber length in Acala and Pima cotton

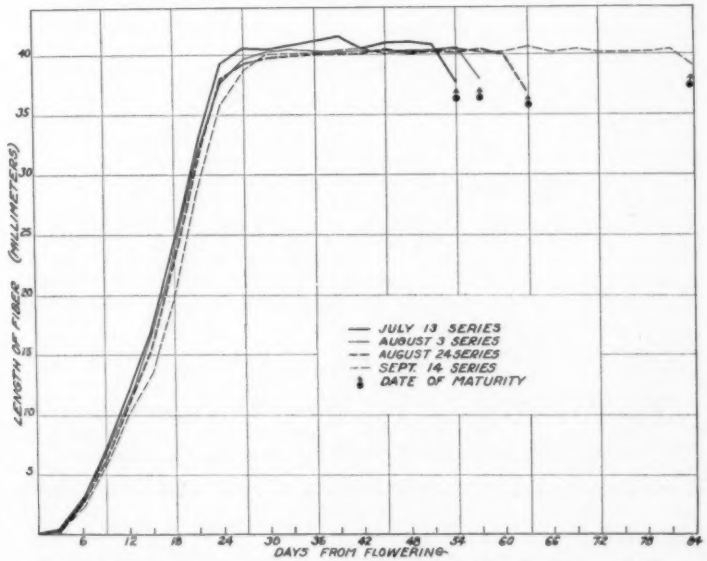

FIGURE 6.—Superimposed curves showing cumulative increase in length of Pima cotton fibers

fibers. The Pima fibers were more regular in length than were the Acala fibers, as indicated by the smaller deviations in length measurements in the former variety. This greater regularity of the Pima fibers became more pronounced as the season advanced, which is evidenced by the wider ratios between mean fiber lengths and the standard deviations in each successive series of the two varieties.

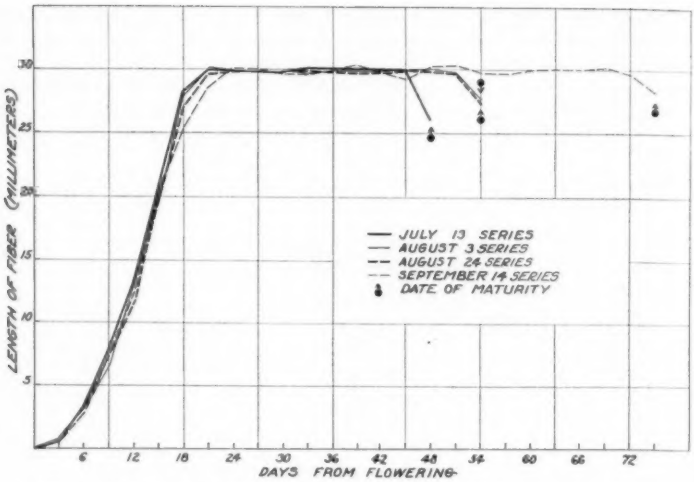

FIGURE 7.—Superimposed curves showing cumulative increase in length of Acala cotton fibers

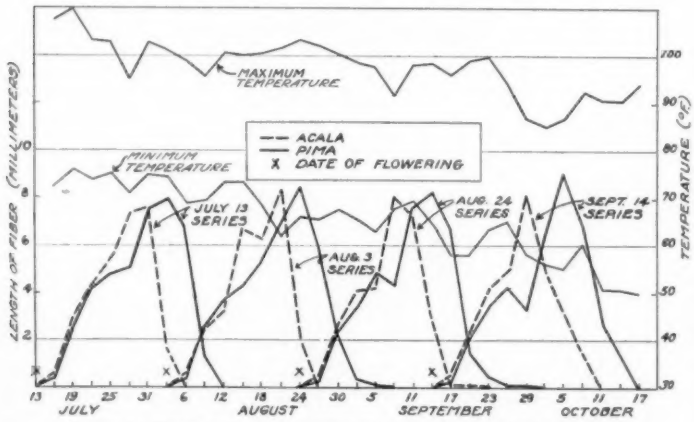

FIGURE 8.—The rate of increase in cotton fiber length as affected by temperature

TABLE 3.—*Development of fiber-wall thickness in Pima cotton from flowering to maturity*

Age of bolls (days)	Thickness of fiber wall in—				Age of bolls (days)	Thickness of fiber wall in—			
	July 13 series	Aug. 3 series	Aug. 24 series	Sept. 14 series		July 13 series	Aug. 3 series	Aug. 24 series	Sept. 14 series
	Microns	*Microns*	*Microns*	*Microns*		*Microns*	*Microns*	*Microns*	*Microns*
6	0.30	0.35	0.35	0.40	57		2.80		
12	.32	.35	.35	.40	60			2.50	2.51
18	.35	.35	.36	.40	63			2.40	
24	.90	.70	.43	.40	66				2.79
30	1.85	1.02	.86	.51	72				2.65
36	2.40	1.90	1.70	.81	75				
42	2.75	2.55	2.00	1.55	78				2.80
48	2.80	2.90	2.37	1.80	84				2.80
54	3.10	2.80	2.35	2.56					

TABLE 4.—*Development of fiber-wall thickness in Acala cotton from flowering to maturity*

Age of bolls (days)	Thickness of fiber wall in—				Age of bolls (days)	Thickness of fiber wall in—			
	July 13 series	Aug. 3 series	Aug. 24 series	Sept. 14 series		July 13 series	Aug. 3 series	Aug. 24 series	Sept. 14 series
	Microns	*Microns*	*Microns*	*Microns*		*Microns*	*Microns*	*Microns*	*Microns*
6	0.36	0.40	0.35	0.40	57				
12	.35	.35	.35	.40	60				2.82
18	.36	.40	.35	.40	63				
24	.63	.70	.52	.52	66				2.83
30	1.64	1.02	1.22	1.12	72				3.18
36	2.28	2.25	1.65	1.43	75				3.20
42	2.58	2.50	2.00	1.82	78				
48	2.75	2.80	2.70	2.10	84				
54		3.12	2.40	2.76					

The cumulative increase in fiber-wall thickness is shown in Tables 3 and 4 and Figure 11. Measurements were taken on material collected at 6-day intervals beginning six days after flowering and continuing until maturity. No appreciable thickening occurred during the first 18 days after flowering with either variety of cotton in any series. The fiber walls began to thicken sometime between the eighteenth and twenty-fourth day in the first two series and to a somewhat lesser extent in the third series. A small amount of thickening also took place in the September 14 series of Acala between the eighteenth and twenty-fourth day, but the walls of the pima fibers did not thicken until after the twenty-fourth day in this last series. The temperatures were declining rapidly during the time the fiber walls were thickening in the August 24 and September 14 series, the maximum temperatures dropping below 75° and the minimum below 40° before thickening was completed in the last series. These temperatures were evidently sufficiently low to inhibit the rapid thickening of the fiber walls. A comparison of Figure 5 with Figure 11 shows that during the period immediately succeeding flowering, elongation of the fibers was proceeding rapidly, but thickening of the fiber walls did not begin until elongation was almost completed. The rate of thickening was slow at first, but became considerably accelerated after elongation had been completed.

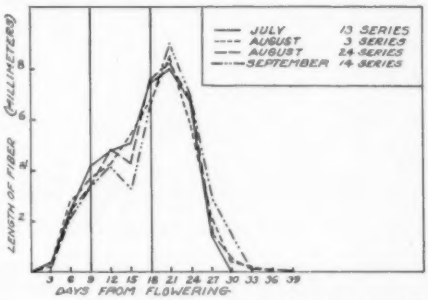

FIGURE 9.—Superimposed curves showing the rate of increase in length of Pima cotton fibers

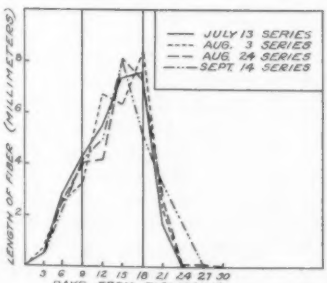

FIGURE 10.—Superimposed curves showing the rate of increase in length of Acala cotton fibers

The curves showing the development in thickness of fiber walls have been superimposed in Figures 12 and 13. Maximum fiber-wall

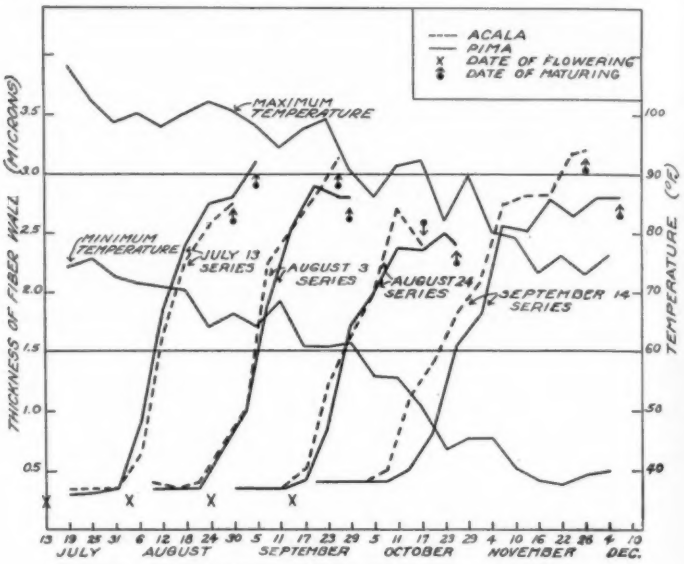

FIGURE 11.—Cumulative increase in fiber-wall thickness in Acala and Pima cotton

thickness was attained in the Pima variety 54, 48, 60, and 78 days after the flowering period in the July 13, August 3, August 24, and September 14 series, respectively. Acala cotton required 48, 54, 48, and 75 days for maximum fiber-wall thickening in these respective series. With the exception of the August 3 series, the Pima fibers required from 3 to 12 days longer for wall thickening than did the Acala fibers.

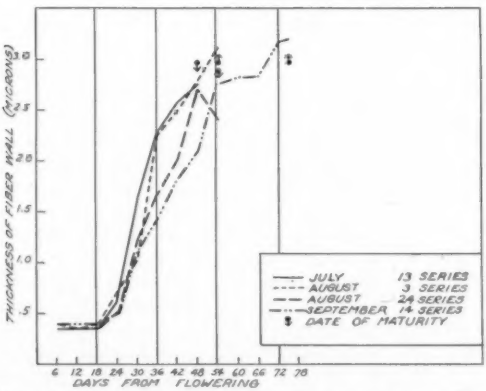

FIGURE 12.—Superimposed curves showing the cumulative increase in fiber-wall thickness of Pima cotton

The effect of temperature on the rate of increase in fiber-wall thickness is well shown in Figure 14. The rate of increase in thickness dropped as the temperatures declined with each successive series with the one exception of the August 3 series of Acala fibers. No explanation will be advanced as to why two high peaks with an intervening low rate of thickening occurred with the Pima fibers in the

September 14 series and also in the August 24 and September 14 series of Acala fibers.

More than one-third of the total wall thickening of the first two series of Acala fibers was acquired during the 3-day period of most rapid thickening. Considerably less than this amount of wall thickening was acquired during the corresponding periods of the last two Acala series. The amount of wall thickening in the Pima fibers during the time of their most rapid thickening was somewhat less than for the Acala in the first two series and more than the Acala in the last two series.

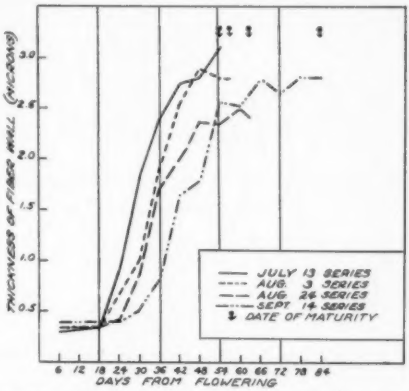

FIGURE 13.—Superimposed curves showing the cumulative increase in fiber-wall thickness of Acala cotton

The superimposed curves in Figures 15 and 16 emphasize differences in the rate of increase in fiber-wall thickness which prevailed between the various series. A comparison of these two figures with Figures 9 and 10 shows that differences between series in the rate of increase in fiber-wall thickness were much more pronounced than were

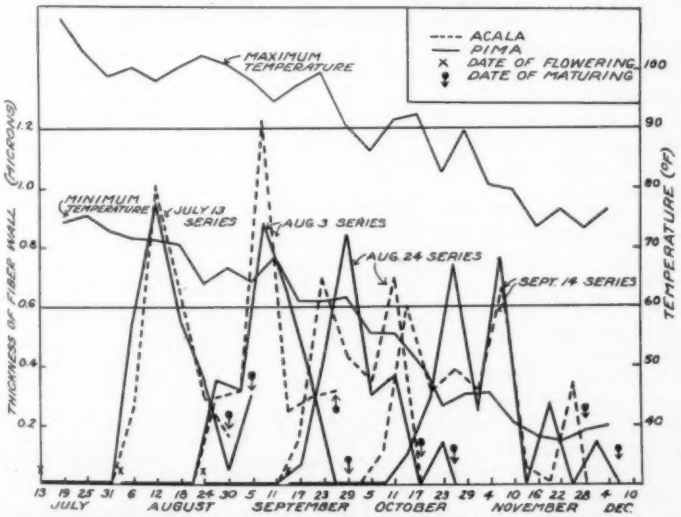

FIGURE 14.—The rate of increase in fiber-wall thickness in Pima and Acala cotton as affected by temperature

differences in the rate of fiber elongation. This was undoubtedly due to the fact that fiber elongation occurred first and was completed in most of the series before temperatures had declined to any great extent.

Fiber-wall thickening was completed at the date of maturity of the bolls in three of the Acala series and in one of the Pima series, while wall thickening was completed in the remaining series from three to nine days before the bolls opened. These differences were due in part to the difficulty in deciding when a boll is mature. The tendency toward delayed opening of the bolls was greater during the latter part of the season and was probably due to the general slowing up of plant activity.

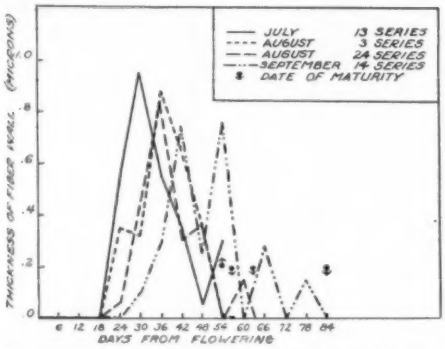

FIGURE 15.—Superimposed curves showing the rate of increase in fiber-wall thickness of Pima cotton

SUMMARY AND CONCLUSIONS

Fiber growth begins at the time of flowering irrespective of fertilization and proceeds rapidly after fertilization but ceases within a few days in unfertilized bolls.

Elongation of Acala fibers was completed 21 days after flowering in the series which flowered July 13, August 3, and August 24; and 24 days after flowering in the series which flowered September 14.

Pima cotton required 27 days for the elongation of the fibers in the first three series and 30 days for those in the September 14 series.

Lower temperatures probably caused the prolongation of the time needed for completion of fiber length in the September 14 series of both varieties.

The fibers of both varieties made a daily increase in length of from three thirty-seconds to one-eighth inch at the time of their most rapid growth.

The greatest increase in fiber length occurred about the twenty-first day after flowering in the Pima series and from the fifteenth to the eighteenth day in the Acala series.

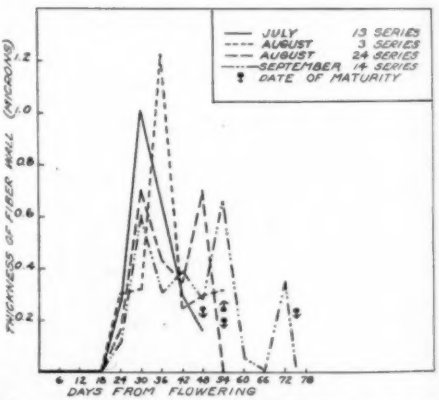

FIGURE 16.—Superimposed curves showing the rate of increase in fiber-wall thickness of Acala cotton

No appreciable thickening of fiber walls began until fiber elongation was almost completed.

The rate of fiber-wall thickening became less, with one exception, in each successive series as the temperatures declined.

Fiber-wall thickening was completed in the Pima fibers 54, 48, 60, and 78 days after the flowering period in the July 13, August 3,

August 24, and September 14 series. Acala cotton required 48, 54, 48, and 75 days for maximum fiber-wall thickening in these series.

Fiber-wall thickening was completed in some instances at the time of boll maturity and in others a few days before maturity.

The time of the season during which cotton fibers are developing affects the rate of fiber-wall thickening greatly but does not influence the rate of fiber growth in length to any appreciable extent until late in the season.

Prevailing temperatures contribute to the rate of fiber development, and when lower than necessary for optimum plant growth, have a retarding effect on both fiber elongation and fiber-wall thickening.

Varietal differences in the development of length and wall thickness of cotton fibers as unlike as Acala and Pima are noteworthy.

SOURING OF FIGS BY YEASTS AND THE TRANSMISSION OF THE DISEASE BY INSECTS[1]

By PANOS D. CALDIS,[2 3]

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INTRODUCTION

Souring was the first disease of the fruit of the fig to be reported from California. For a long time it was the only disease recognized and the term was used indiscriminately to cover all kinds of spoilage. The first reference to this disease was made by Pierce, as reported by Galloway (*11 p. 239–240* [4]) in 1892. He states:

> But another one of the industries of the State which has been greatly extended of late is seriously threatened. This is the growth and curing of figs. It has been observed since the cultivation of this fruit has been attempted that the grower had to contend with a destructive fermentation of the fruit which often caused the loss of nearly the entire crop.

Pierce found that the fruits spoiled, both while on the tree and on the drying trays and that the causal agent was a yeast.

> Numerous experiments with powders and sprayers were used on the trees but with entirely negative results. The cause probably lies in the fact that the fruit is inoculated by insects, the yeast cells being carried by them to the ripening fruit.

No further results were published by Pierce. Howard (*18, p. 93–94*) in 1900 refers to souring in the Smyrna fig (Calimyrna) as follows:

> Souring of the figs was not noticed in the early part of the season, but began later to a limited extent when showers occurred. When the Smyrna figs ripen the ostiolum opens wide and remains open so that a match can easily be inserted and often moderate-sized insects can enter and feed on the sugar. Some of them are caught in the sticky sap and die within the fig. When the figs are ripe and fall ants and beetles of the genera Noxtoxus and Carpophilus enter in this way.

Roeding (*27, p. 50*) in 1903 studied the fig at home and abroad and mentioned souring as occurring in Asia Minor and in California.

> Experience has shown, however, that the Smyrna varieties suffer far less from this trouble than the ordinary sorts. In the orchard of the Fancher Creek Nurseries, where a few of the White Adriatic figs are still growing, from 50 to 75 per cent will sour on the trees, and in adjoining rows of Smyrna figs it is only occasionally that a sour fig can be found.

Eisen (*8*) and Rixford (*26*) also mention souring in an inclusive way. Rixford found tht a closed eye prevents fermentation. Coit and Johnston, as reported by Haring (*17*) in 1921, did not find a specific organism responsible for the decay of figs observed. "Many types of rot were observed, from the soft, watery, fermented type to the typical dry-rot type. Various yeasts, fungi, and bacteria seem to be responsible."

[1] Received for publication Nov. 5, 1929; issued May, 1930.

[2] Most of the work was done while the writer was the recipient of the James Rosenberg memorial scholarship in agriculture.

[3] The writer wishes to acknowledge his indebtedness to Prof. Ralph E. Smith of the University of California, under whose direction the investigation was carried on, for suggestions and criticisms, and for critical reading of the manuscript.

[4] Reference is made by number (italic) to Literature Cited, p. 1049.

The writer commenced work on this problem in the fall of 1922. It immediately became evident that the decay commonly given the name "souring" was not caused by one agent alone, and that the symptoms were obvious enough to allow the division into at least two distinct diseases. It was furthermore found that the symptoms on caprified varieties, such as the Lob Ingir, Stanford, San Pedro Black, and several unnamed seedlings requiring caprification, were rather of the character of a rot than of a fermentation and were found solely on caprified figs. While fermentation also occurred on caprified

FIGURE 1.—Sour Adriatic fig with jellylike exudate coming out of the eye which indicates that active fermentation is taking place within the fruit

figs, alone or at the same time as the rot, figs of parthenocarpic varieties such as the Adriatic, Mission, Kadota (Dottato), and spring crop San Pedro Black, never exhibited the rot. The findings of the writer on this rot of the fig, termed "endosepsis," have already been discussed (*5*). It is the purpose of this paper to discuss the etiology and transmission of souring.

SYNONYMY AND SYMPTOMS

Souring, as previously mentioned, is the name commonly used to describe all forms of fig spoilage. The term should be restricted to the symptoms described below to cover the spoilage due to fermenta-

tion organisms. Fig fermentation would be a more appropriate name for this disease, but the term "souring" is well established and, when restricted to the disease under discussion, is not confusing.

The symptoms are best observed on fruit of parthenocarpic varieties that have not been caprified, such as the Adriatic. In Lob Ingir and other caprified figs the symptoms are liable to be obscured or confused with the symptoms of endosepsis, or internal rot, that attacks only caprified figs. The symptoms of the disease are manifested only when

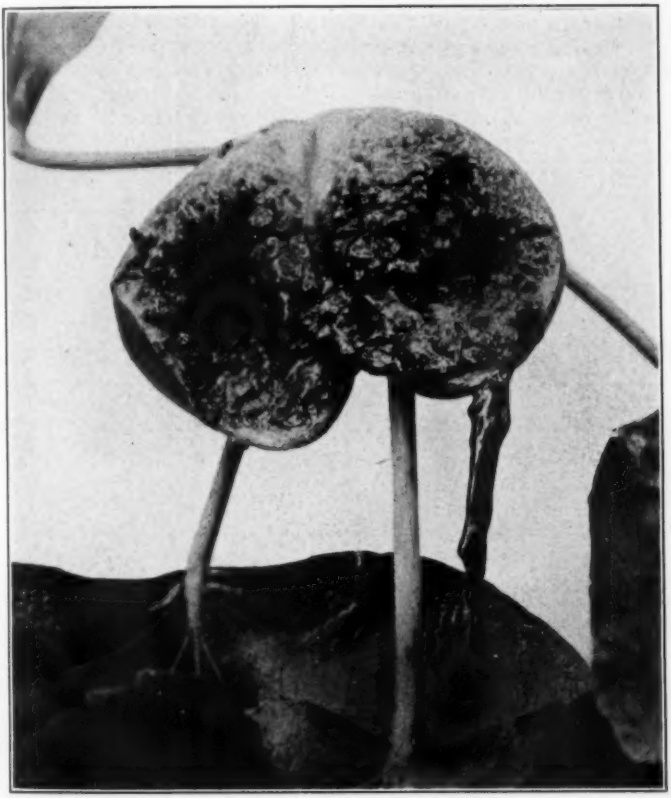

FIGURE 2.—Interior of sour fig shown in Figure 1

the figs begin to ripen and the eye is wide open. In no case has any deterioration been observed in figs of parthenocarpic varieties before the eye opens. Philips et al. (*23*) and Caldis (*5*) have found that the pulp of such figs is sterile previous to the opening of the eye and for considerable time afterwards.

In souring there is at first a change in the color of the pulp, which from pink becomes colorless, and subsequently turns watery. A pink liquid exudes through the eye (fig. 1), dropping on the leaves or jelly-

ing at the eye. Gas bubbles are seen through the pulp and in the skin, and in many cases the pulp becomes water-soaked and loses its firmness. The pulp is disintegrated and smells strongly of alcohol (fig. 2), and is often found to be covered by a white scum. (Fig. 3.) In this condition the figs begin to shrivel and dry up, either dropping to the ground or hanging on the twig, in the latter case giving rise to what is commonly called "black neck" figs. A dead spot or "eye canker" is often formed in the bark at the point of attachment of such figs, as seen in the two twigs at the top of Figure 4. Fermented figs lose their firmness and sag, and usually the pulp becomes detached from the skin at the neck which shrivels, dries up, and turns dark.

Fig souring is primarily an alcoholic fermentation, but subsequent changes may take place while the fig is attached to the twig, on the ground or on the drying board. The commonest change is the action of acetic bacteria on the alcohol with the production of acetic acid,

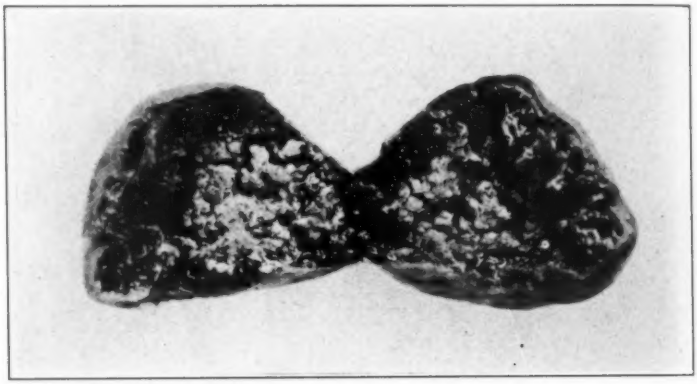

FIGURE 3.—Yeast scum sometimes found inside sour figs

which is readily discerned by its pungent, strong odor. Ethyl acetate and other esters are probably formed.

DISTRIBUTION AND ECONOMIC IMPORTANCE

Souring has been observed wherever the fig is grown in California. No section is free from it, although orchards have been observed with a very small percentage of figs suffering from this disease. Estimates as to the percentage of injury can not be accurate in the case of Lob Ingirs on account of the coexistence of endosepsis; however, in the case of Adriatics, which are not caprified, souring is very abundant in certain seasons and certain localities and at times increases the cull pile to include the entire crop. There have been cases known to the writer where the crop has not been gathered at all on account of this disease. A grower of Adriatics in the San Joaquin Valley estimated the loss from souring in 1923 as 80 per cent. Such losses are common. Indeed, Adriatic figs seldom escape a high percentage of spoilage from this cause. The reasons for variations from year to year and from locality to locality can best be discussed when the facts regarding transmission have been given. The influence of

the environment will be taken up also at the time, as well as varietal susceptibility.

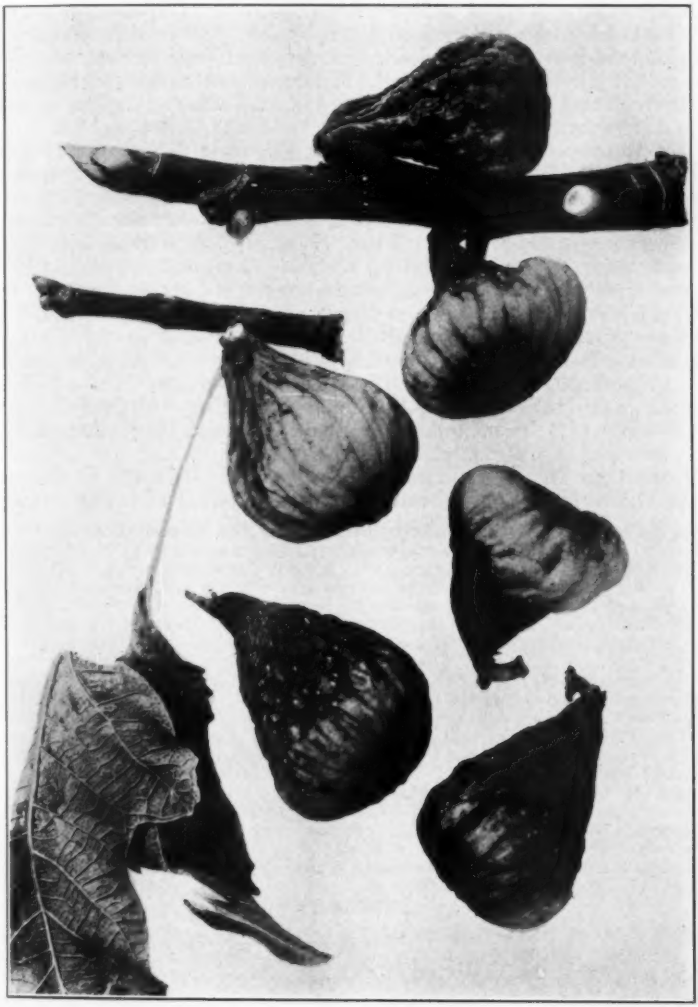

FIGURE 4.—Dried, sour, "black neck" figs, some of which are still attached to twig; note also dead spot or "eye canker" formed in the bark at point of attachment of such figs

This disease has been observed in foreign fig-growing countries by several travelers, but there is no reference in literature as to importance and extent of damage. Letters of inquiry were written to the departments of agriculture of Italy, France, Greece, Spain, Jugoslavia, Portugal, Turkey, and South Africa in the fall of 1922.

Replies were received only from Italy, Spain, and South Africa. G. B. Traverso, director of the Royal Station of Vegetable Pathology, Rome, Italy, stated that the disease "does not exist in Italy." The director general of agriculture and forestry of Spain replied that "the disease probably exists in Spain * * * but there have been few figs seen with these characteristics, and they never constitute an epidemic" I. Tribolet, of the division of horticulture, department of agriculture, Union of South Africa, stated that he had never seen the disease in that country.

No mention of souring is made by Edgerton (*7*), Gould (*12*), or Matz (*21*) in their studies of fig diseases in the South Atlantic and Gulf States. Siniscalchi (*32*), De Rosa (*28*), Portale (*24*), and Guglielmi (*13*) make no mention of souring among the fig diseases enumerated as occurring in Italy. Ferrari (*10*), writing about the fig industry in Cosenza, Italy, describes a disease of the fruit in connection with a bacteriosis of the tree. He states that the figs attacked by the disease when nearly ripe show a drop of liquid at the eye, first yellow, then reddish, which increases in volume and, if the infection is heavy, is exuded. This may be souring, but there are no definite data given. Vallese (*36*) also mentions a disease of the fig in Italy that may be similar to souring. Trabut (*35*) and Guillochon (*14*) from North Africa and Esterlich (*9*) from Spain do not mention souring at all in their papers.

Condit, in an unpublished report on the fig industry in Europe, Asia Minor, and north Africa, states the following about fig souring:

> In my report on my trip to Europe I find the following regarding fig souring: The fact undoubtedly is that fig souring occurs more commonly than the Europeans like to admit. The fancy Smyrna layer or Locoum figs, the Greek string figs, or the Spanish fleur figs, which are seen in city markets, represent only the best part of the crop. For example, fig merchants of Smyrna prefer the crop from the hillside orchards of the upper Meander Valley because the figs in the Sokia district and especially in the Ayassouluk or coastal district are soft, sour, dark colored, and inferior. It is a well-known fact that the largest proportion of the fig crop of Mallorca Province, Spain, is used for hog feed or distilled on account of its poor quality. The packers of Coin, Malaga Province, Spain, state that the growers deliver only 50 per cent of the crop to the packing house, feeding the other inferior half to cattle and hogs. Immense quantities of sour and rotten or inferior figs are shipped from Southern Italy to Trieste or Vienna for coffee factories. I myself found Smyrna figs souring at Ayassouluk on August 20, but not in serious quantities. At Kalamata figs of a garden variety were souring badly in the yard of Mr. Pantaxopoulos near the seashore on September 4. Rain-damaged figs were very abundant throughout southern Europe this season, but these could hardly be classed as sour figs. The lateness of the season in Spain and Portugal prevented actual observations along this line.

ETIOLOGY

FORMS OF THE YEASTS ISOLATED

Pierce (*11, p. 240*) isolated

> a yeast, which when pure cultures were made, was applied to fruit on both trees and the drying board. The result was the production of an exactly similar fermation to that occurring naturally.

No identification of the organism is reported. Coit and Johnston (*17*) could not assign a specific organism to the decay. They found various yeasts, fungi, and bacteria apparently responsible for the disease.

When fruit not affected with endosepsis was examined and showed the symptoms described previously, especially the early ones, the

author has invariably isolated pure cultures of yeasts. The disease was studied mostly on figs of the Adriatic variety. This variety is very susceptible to souring, it is widely planted in California and, when it is not caprified, endosepsis does not affect it. There is more than one species of yeast responsible for this disease. It is conceivable that every true yeast introduced into the cavity of the fig, when the latter is ripe, the cavity full of sugary solution and the concentration not too high, would be capable or exciting fermentation. In many platings of sour Adriatics, however, it was found that principally two forms of yeasts were invariably present. A third one was also present but was found incapable of producing the disease. All three forms belong to the class of asporogenous or wild yeasts. The form or forms most often found is a top yeast that produces a scum in liquid media and in the fig cavity and belongs most probably to the Mycoderma class. (Fig. 5.) The second form often present is a bottom yeast, growing poorly on artificial media, especially liquid ones, and belonging to the Apiculate class. The third form is a round yeast of the Torula type. These forms are hereafter designated as A (Mycoderma), C (Apiculate), and E (Torula). Single-cell isolations of these yeasts made by the micropipette method were used in inoculations and in studying their fermentation and other physiological activities.

CULTURAL CHARACTERISTICS OF THE YEASTS ISOLATED

Two Per Cent Dextrose Broth

Yeast A: A thin veillike pellicle is produced on the surface of the liquid in 24 hours and climbs up the sides of the tube. The liquid is cloudy with an abundant sediment. Copious gas is evolved on shaking the tube.

Yeast C: A scarcely noticeable precipitate is formed at the bottom of the tube. This yeast grows very poorly, if at all, in the liquid media used, except in grape juice. A brownish pigment is produced at room temperature (21° C.) and at 28° C. No pigment at lower temperature.

Yeast E: Cloudiness and abundant precipitates are produced, but no pellicle. A small amount of gas produced by some strains.

Two Per Cent Dextrose Nutrient Agar

Yeast A: An abundant, spreading, dull white, somewhat powdery growth is produced, the margin is lobate, with ciliate edge more evident on plates. (Fig. 5.) The center of the stroke is raised, whereas the margins are filmlike.

Yeast C: Very scanty, wet, transparent, beaded growth is produced, with a brownish pigment at room temperature.

Yeast E: Abundant, spreading, white, shiny butyrous growth, with smooth margin.

Malt Agar [5]

Yeast A: Abundant growth, dull white, less than on dextrose nutrient agar.

Yeast C: Scanty whitish growth.

Yeast E: Abundant shiny white growth.

[5] One hundred grams of germinated barley was ground up and 1,000 c.c. of water added. The mixture was heated at 55° to 58° C. for one hour and held over for 24 hours. It was then boiled and filtered, agar added, was tubed and sterilized.

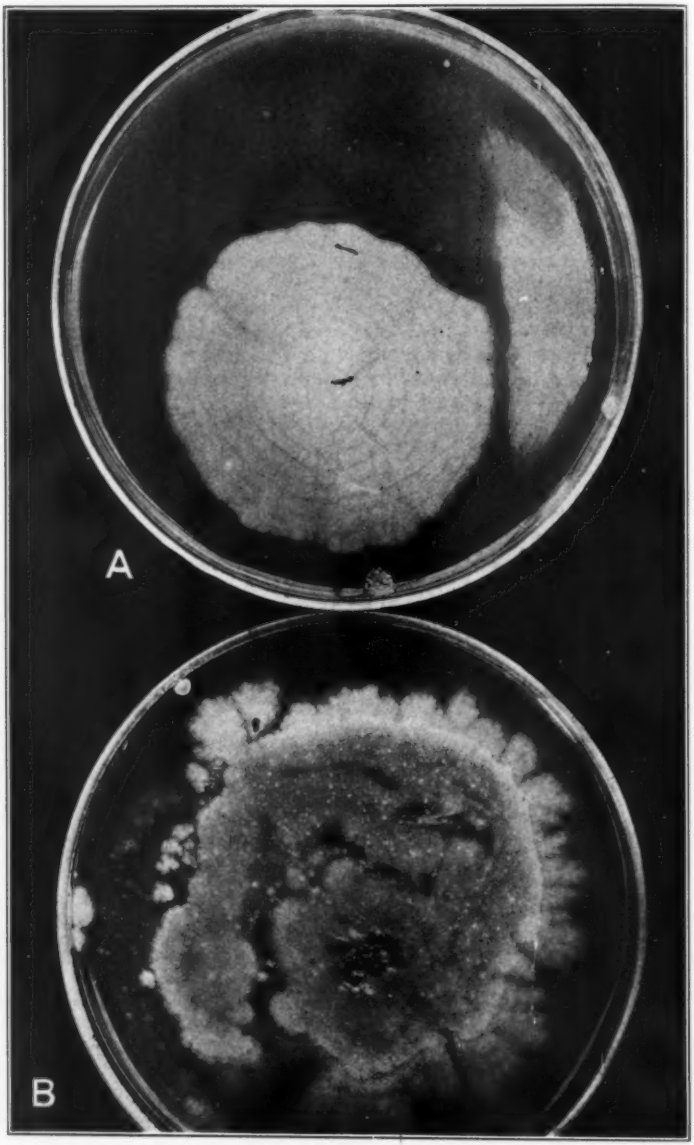

FIGURE 5.—Cultures of yeast A obtained (A) from dried-fruit beetle and (B) from the internal tissue of a sour fig

FOUR PER CENT DEXTROSE LAURENT LIQUID [6]

Yeasts A and E: As in dextrose bouillon.
Yeast C: No growth.

GIANT COLONIES ON TWO PER CENT DEXTROSE NUTRIENT AGAR PLATE

Yeast A: Growth rapid, irregular, dull white, with rough surface, effuse elevation, margin lobate, cilliate. (Fig. 5.)
Yeast C: Slow growth, round to irregular, with flat, smooth surface, undulate to lobate edge.
Yeast E: Medium growth, round colony, three-fourths of an inch in diameter, white, shiny, elevation effuse, surface ringed, edge entire.

LAURENT AGAR PLATE

Yeast A: Large irregular colonies, dull white.
Yeast C: Small transparent white colonies.
Yeast E: Small, shiny white round colonies.

FIG INFUSION [7]

Yeast A: Thin pellicle, liquid clear, brownish sediment, thick ring.
Yeast C: Slight ring, no pellicle, brown sediment.
Yeast E: As in A.

FERMENTATION OF SUGARS BY THE YEASTS ISOLATED

Glucose, fructose, sucrose, lactose, and maltose have been tested, as well as apple cider, grape juice, and fig infusion. The sugars were added either to beef bouillon, to Laurent solution, or to the ammonium phosphate medium used in sugar fermentation with bacteria (*33*). Smith and Dunham fermentation tubes were used.

Nine isolations of yeast A, three of yeast E, and four of yeast C were inoculated into Smith fermentation tubes containing 2 per cent dextrose, fructose, sucrose, maltose, lactose bouillon, cider, Zinfandel grape juice, and fig infusion.

It is evident from Table 1 that the disaccharides are not fermentable by the three yeasts isolated from figs. Gas was produced from dextrose and fructose to a great extent by yeast A. Yeast C produced a small amount of gas from fructose 30 days after inoculation. Yeast E also produced a small amount. Gas was produced abundantly by the three yeasts from grape juice, and by yeasts A and C from fig infusion, but not by yeast E. Pellicle, a cloudy bulb, but a clear arm was produced by yeast A on all the above media. Maximum gas was produced by yeast A on all the media. Maximum gas was produced in 10 to 12 days. The nine isolations of yeast A produced variable percentages of gas from dextrose in the closed arm of the fermentation tube. The percentages varied from 4.7 to 23.5 per cent, an average of 14.4 per cent produced in from 5 to 8 days. The greater percentage of gas was produced in 8 days, the next (23.5 per cent) in 5 days, while the lowest amount of 4.7 per cent was produced in 10 days.

[6] Composed of 4.71 gms. ammonium sulphate, 0.75 gm. potassium phosphate, 0.1 gm. magnesium sulphate, 1,000 c. c. water and 2 per cent of the sugar to be studied.
[7] One hundred grams of dried figs boiled in 1,000 c.c. of water for one-half hour, filtered and sterilized. Agar added, if wanted.

TABLE 1.—*Results of fermentation tests of various sugars and fruit juices with yeasts obtained from sour figs*

Medium	pH	Yeast A	Yeast C	Yeast E
2 per cent dextrose broth	6.4	Abundant gas	Slight growth, no gas	Small amount gas, slight growth.
2 per cent fructose broth	6.4	do	Slight amount in 30 days	No gas.
2 per cent sucrose broth	6.6	No growth	No growth	Small amount gas.
2 per cent maltose broth	6.5	do	do	No growth.
2 per cent lactose broth	6.5	do	do	Do.
Apple cider	3.4	Small amount gas	do	Do.
Zinfandel grape juice	3.9	Abundant gas	Abundant gas	Abundant gas.
Fig infusion	4.8	do	do	No gas.

Zinfandel grape juice was found to be a favorable medium for all these yeasts. To test their fermentation abilities, 200 c. c. of grape juice was placed in 500 c. c. Erlenmeyer flasks and sterilized by steaming for three consecutive days. They were then inoculated in duplicate with three single cell isolations of yeast A made by the micropipette method, one of yeast C, and one of yeast E. The flasks were weighed daily, and when the loss of weight was comparable to that of the check, a portion of 100 c. c. was distilled with the addition of 50 c. c. of water. The three strains of yeast A produced an average of 7.10 per cent of alcohol by volume (4.7 per cent by weight). Yeast C produced 8.22 per cent and yeast E 6.55 per cent (6.6 and 5.5 per cent, respectively, by weight).

For another test of the fermentation and acid production by yeasts A and E, ammonium phosphate medium and beef-extract broth, both solid and liquid, with the addition of brom cresol purple indicator, were used with each of the following sugars: Dextrose, sucrose, and lactose. The solid media were slanted, the liquids were put in Dunham fermentation tubes. Yeast A produced both acid and gas from the dextrose synthetic, and gas but no acid from the dextrose bouillon. Neither acid nor gas was produced from either the liquid or solid, the synthetic, or the bouillons with the addition of sucrose and lactose. Yeast E produced acid on the liquid synthetic dextrose, but no gas. A small amount of gas, but no acid, was produced after 13 days from dextrose bouillon. Acid and gas were produced from sucrose, both in the synthetic and the broth media. Neither acid nor gas was produced from lactose in either type of medium. The production of gas by yeast E was found to be irregular. Of the three isolations made, which otherwise appear identical, one produced small amounts of gas from glucose and grape juice, the other two did not.

As mentioned previously, the yeasts isolated from figs act indifferently toward disaccharides. It is of interest that figs do not contain such sugars. Leclerc du Sablon (*30*) found very little nonreducing sugars in the varieties Dorée (Figue d'or), Datte Quodidienne (Figue datte), and Barnissotte Black (Bourjasotte noir). An analysis for sugars of healthy and diseased Adriatic figs was made by the writer in 1923. Healthy figs picked fresh contained 69.61 per cent of reducing sugars soluble in alcohol, while sour figs, picked as they dropped from the tree, contained 59.45 per cent of reducing sugars soluble in alcohol, both percentages calculated on a moisture-free basis. No nonreducing sugars were found in either the healthy or the diseased figs.

To demonstrate that this disease is actually a fermentation, the following experiment was tried: Sour Adriatic figs were selected from

the drying board and examined for the typical symptoms of the disease. Eight hundred grams of the figs were macerated with 500 c. c. of water and steam-distilled for three hours. The first portion of the distillate was neutralized with potassium hydroxide and redistilled. Fifty seven cubic centimeters of distillate was obtained, with a specific gravity of 0.9812, or 13 per cent of alcohol by volume. The second fraction was also neutralized and redistilled, 250 c. c. was obtained, with a specific gravity of 0.9950, or 2.20 per cent of alcohol by volume. Sour Adriatic figs picked from the ground as they were dropping from the trees, were treated as above and 3.55 per cent of alcohol by volume was obtained. A peculiar, ethereal odor passed into the distillates.

Rossi (*29*), in his extensive review of the literature regarding apiculate yeasts, mentions that *Pseudosaccharomyces apiculatus* isolated from grapes produces 3.15 per cent of alcohol by weight from dextrose. He states that acetic and formic acid as well as esters and other volatile substances were found by Amthor, Müller-Thurgau, Kayser, Seifert, and Mach e Portele to be produced by this yeast. It has been mentioned already that sour figs at times have an ester smell.

MORPHOLOGY OF THE YEASTS ISOLATED

Yeast A: A top yeast with long and narrow cells varying considerably in length. Budding apical, the daughter cells remaining attached to form long chains with as many as three daughter cells attached to the same apex. A few cells are slightly curved. The cells are vacuolated, especially on solid media. No spores are produced on plaster blocks, Gorodkowa's medium (*20*) or carrot plugs. The cells are 6 to 33 by 2.2 to 6 μ (average 8.9 by 3.9) on dextrose nutrient agar and 7.5 to 31.5 by 3 to 6 μ (average 12.4 by 4.7) on dextrose bouillon. (Fig. 6.)

Yeast C: Typically apiculate bottom yeast measuring 4.1 by 2.0 μ, single or in pairs, sometimes elliptical, never in threads. No spores found. (Fig. 6.)

Yeast E: A bottom yeast with almost perfectly round cells, with a large vacuole, budding freely, the daughter cells remaining attached for some time forming chains of three or four cells. No spores are produced on plaster blocks, Gorodkowa medium or carrot. Budding cells measure 4.19 by 3.4 μ and 3.9 by 3.9 μ when not budding. Some strains are smaller, measuring 3.1 by 3.1 μ. (Fig. 6.)

PATHOGENICITY OF THE YEASTS ISOLATED

Inoculations with single-cell cultures of these yeasts were made on fresh figs that were still attached to the tree. Figs were selected with the eye fairly well closed and inoculated by inserting a needle through the eye.

The whole twig was then bagged with a 3-pound manila paper bag and tied firmly in order to exclude insects from the fig. (Fig. 7.) Inoculations were made in triplicate. The eyes of the figs inoculated were previously swabbed with alcoholic mercuric chloride. The figs when ripe were brought to the laboratory and plated.

Yeast A: Typically sour figs were obtained by inoculating with this yeast. The pulp of the figs was discolored and covered by a white scum. One side of the fig was softened and water-soaked. The figs were often bloated, gassy, and sagging, with the pulp drawn away from the stem end. Slight dripping was observed. When green figs were inoculated, the results were sometimes negative.

Yeast C: Fermented figs were obtained with this yeast essentially as with yeast A, but without the scum on the surface of the pulp cavity.

Yeast E: Inoculations with this yeast did not yield typically sour figs. The pulp of the fig was discolored and gelatinized with an odor approaching that of sour figs.

TRANSMISSION

Berlese (*2*, *3*, *4*) has found that yeasts are very scarce in the air during late spring and early summer, but abundant on trunks and

FIGURE 6.—Yeasts isolated from sour figs. Camera lucida drawings × 1,400 from 7-day old cultures. Yeasts A and E grown on Laurent liquid, C on dextrose nutrient agar. A', pellicle of yeast A on dextrose bouillon

other parts of trees. He found *Saccharomyces apiculatus*, *S. ellipsoideus*, and *S. pastorianus* in the soil from April to June, but rarely afterwards. Insects, however, such as ants and flies (*Sarcophaga carnaria* L., *Drosophila cellaris* L., *Calliphora erythrocephala*, Dasyphora, Crysotoxum, Eristalis, Aricia, Lucilia, and *Anthomyia* sp.),

were found carrying yeasts both externally and internally. Yeasts were found to multiply in their intestines and to hibernate in them.

Phillips et al. (*23*) in their studies on fig smut found that exclusion of insects from two large fig trees by building a tent of muslin cloth over the trees reduced the amount of souring to nothing, while there was a large number of sour figs on the neighboring trees. They also found that the insects most common in figs still on the tree were the dried-fruit beetle, *Carpophilus hemipterus* L., and the vinegar fly, *Drosophila ampelophila* Löw. It may be deduced, therefore, that these two insects may be concerned in the transmission of the yeasts causing souring into the cavity of the fig. In order to test this theory, a number of sacks were made of brass strainer cloth 1 by 1.5 feet,

FIGURE 7.—Figs covered with paper bags to exclude insects in experiments on the transmission of souring by inoculation with yeasts

soldered on top and side. These sacks were slipped over fig twigs bearing from 3 to 9 figs, fully grown but green, whose eye scales were tightly closed. The sack was sewed at the bottom, and a large plug of cotton placed in the opening through which the twig was introduced. One hundred manila paper sacks were also placed on similar figs. (Fig. 7.) Adriatic figs were used in this experiment in 1924; six strainer-cloth sacks were placed on August 1 and six more on August 26. When the figs commenced ripening in the sacks (September 8) 12 dried-fruit beetles, collected from figs in sterile vials by holding the mouth of the vial over the eye of the fig and tapping the sides so that the beetles emerged from the fig and entered the vial, were introduced into each of four of the sacks. The beetles were watched awhile, and they were seen crawling on the figs. Many sour figs were on the

trees on which the sacks were placed. When every fig in the sacks ripened and dried up, the twigs bearing the sacks were cut and brought to the laboratory for examination. The results are given in Table 2.

TABLE 2.—*Effect upon souring of exclusion and introduction of Carpophilus hemipterus L. into strainer-cloth sacks inclosing ripening Adrialic figs*

No beetles introduced			12 beetles introduced in each sack				
Sack No.	Total figs	Sour	Sack No.	Total figs	Sour	Wormy but not sour	Sound
1	5	None.	4	7	3	.....	3
2	4	None.	6	9	3	6	.....
3	9	None.	7	5	2	.....	3
5	8	None.	12	5	.....	5	.....
8	4	None.					
9	6	None.	Total	26	8	11	6
10	3	None.					
11	6	None.					
Total	45	0					

The beetles fed and bred in many of the figs, as many more beetles were found in the sacks than the number introduced. Larvae were also found in them. No insects of any kind were found in the eight sacks where they were not purposely introduced. The results indicate that the beetles enter the figs readily but do not cause fermentation unless they are themselves infected. In many cases the wormy figs had been sour, but the alcohol had evaporated in drying, leaving a seedy, dry fig, wormy, devoid of sugar but not sour in the ordinary sense. This has been observed also in the orchard. As mentioned previously, the spoilage may stop at any stage in its development, depending on drying conditions, moisture, and presence of the proper organism.

The beetles from the different sacks were collected and cultured in tubes of dextrose bouillon. The sour figs were plated. The results are as shown in Table 3.

TABLE 3.—*Results of making cultures from Carpophilus hemipterus L. found in sacks containing sour figs*

[Cf. Table 2]

Sack No.	Number of beetles	Number infected with yeasts	Type of yeast
4	3	1	C
6	8	3	A
7	7	7	C
12	8	0	.....

From these experiments it may be concluded that *Carpophilus hemipterus* L. is a transmitting agent of yeasts and bacteria into the figs, where they set up fermentation if the sugar concentration is favorable. To determine the actual flora carried by the dried-fruit beetles, and especially whether the transmission is internal or external, beetles were collected at different times from fig orchards by holding

the mouth of a sterile vial against the eye of an infested fig and tapping the sides. The beetles entered the vial and were thus taken to the laboratory alive and uncontaminated. Drosophila were caught in the same way. The insects were studied as follows: (1) Caught with sterile tweezers and dropped into sterile tubes of dextrose bouillon. One beetle was thrown into each tube. The beetles were either crushed or dropped according as they were dead or alive; (2) placed in Gooch crucibles and disinfected by immersing in 15 per cent solution of potassium hydroxide for 2 minutes, then in 1:500 solution of mercuric chloride for 3 minutes, and washed finally with sterile (autoclaved) water until the washings were free of chlorides as determined by testing with silver nitrate solution; momentary dipping in 95 per cent alcohol was at times used, instead of potassium hydroxide, and 1:1,000 alcoholic (50 per cent) solution of mercuric chloride, instead of the 1:500 solution. No difference was observed in the two methods of disinfection, both being equally effective. After disinfection, the beetles were dropped into tubes of sterile dextrose bouillon, one beetle per tube, left in this tube for varying lengths of time, 1 to 20 minutes, and then removed by means of a sterile platinum loop and dropped into another tube where they were left, The purpose of the shaking into the first tube for varying lengths of time was to obtain a check on the effectiveness of the external sterilization. Unsterilized beetles were shaken into one tube first and transferred into a second tube afterwards in order to determine whether there was any difference between their external and internal flora. Different lots of beetles were tested separately, and beetles were also collected from decaying watermelons, which serve as overwintering grounds for these insects, as was found by Phillips et al. (*23*) and the writer. The results of these tests are summarized in Tables 4 and 5.

TABLE 4.—*Results of various culture tests on Carpophilus hemipterus L., sterilized and unsterilized, as a carrier of yeasts*

UNSTERILIZED

[Figures refer to numbers of insects cultured]

Treatment of beetles	Carpophilus hemipterus L. secured from—																				
	Figs												Watermelons			Figs					
	Aug. 30, 1924			Sept. 12, 1924			Oct. 6, 1924			Oct. 30, 1924			Nov. 11, 1924			Oct. 27, 1925			Nov. 5, 1925		
	Type of yeast																				
	A	C	Negative	A	C	Negative	A	C	Negative	A	C	Negative	A	C	Negative	A	C	Negative	A	C	Negative
Dropped in bouillon and left	2		1	9	3	1	4	8	13	2	4	3	4	9	4	5	6	11	5		23
Left for 1 to 19 minutes, then removed	14		*2	4	2						1	2	1		3		2	2			5
Insects from last			2	1						1		1	1	1	2		3				

STERILIZED

Dropped in bouillon and left (uncrushed)	5		5		5	3					3	6		1	3	1	1	5	1		4
Dropped in bouillon and left (crushed)	4	6	6	4	2	3						8		2	1			13	1		11
Left for 1 to 20 minutes then removed (shaken)			5			9						16			10			24			11
Insects from last	4			2	2	5					3	15	2	3	6		2	22			11

* The insects were sterilized after removing from tube 1.

TABLE 5.—*Summary of Table 4*

UNSTERILIZED

Item	Beetles carrying yeast A		Beetles carrying yeast C		Beetles negative		Total beetles used
	Number	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*	
1. Dropped in bouillon and left	31	26	30	26	56	48	117
2. Left for 1 to 19 minutes, then removed	19	53	5	14	12	33	36
3. Insects from 2	3	21	6	43	5	36	14

STERILIZED

1. Dropped in bouillon and left (uncrushed)	70	16	10	23	26	61	43
2. Dropped in bouillon and left (crushed)	9	15	10	16	42	69	61
3. Left for 1 to 20 minutes, then removed (shaken)					75	100	75
4. Insects from 3	8	10	10	13	59	77	77

The fig tissues yielded the usual yeasts, yeast C and yeast A. In sack 12, where none of the figs soured, the beetles were found to be free of yeasts. Different bacteria were also obtained from the beetles. The figs inclosed in manila paper sacks at the same time as the strainer cloth sacks were also found to be in perfect condition.

This experiment was repeated in 1925 at Davis, Calif., figs of the following varieties being used: Adriatic, Verdal, and Mission, using 20 sacks, which inclosed a total of 147 figs. Of these, 29 figs (3 sacks) were exposed to dried-fruit beetles. Nine of them were found wormy and on plating yielded the usual yeast. The figs were dry and seedy, smelling faintly of alcohol. The remaining 20 figs were not sour. The sacked figs which were not exposed to beetles (17 sacks, 118 figs) were all found to be in perfect condition.

Rand and Pierce (*25*) in 1920 have reviewed the literature on the subject of insect transmission of plant and animal diseases. Insects transmit pathogenes in three ways: (1) Mechanically, by picking up the spores on the exterior of their bodies and accidentally sowing them on the surface or inoculating them into punctures; (2) by making avenues of infection through wounds; and (3) by transmitting them internally, either mechanically or biologically.

The results in Table 4 show that both forms of yeasts isolated from fermenting figs are carried by the dried-fruit beetle. When the beetles were dropped in tubes of broth and left for different intervals of time, 1 to 10 minutes, and then removed, fermentation was set up in many cases (67 per cent), indicating that the types of yeasts mentioned previously are carried mechanically externally. The elytra, the legs, the thorax, and the abdomen were removed and tested separately, and were all found carrying the yeasts. Fermentation was also caused by beetles dropped into bouillon tubes and left uncrushed or crushed. When the beetles were previously sterilized by one of the methods described and then dropped into tubes of bouillon and left uncrushed or crushed, fermentation was also set up. When such beetles were shaken for a limited length of time, 1 to 20 minutes, in a tube of broth and then transferred to another tube where they were left crushed or even uncrushed, there was never fermentation or any kind of growth in the first tube, but in many cases (23 per cent) fermentation was set up in the second tube, indicating that transmission is also internal mechanical. No attempt was

made to discover whether the transmission is internal biological, i. e., whether the yeasts actually multiply in the intestines of the beetles. Berlese (*4*) has shown this to be the case with flies. Both types of yeasts were found to be carried at the same time, and in one case one yeast was found carried externally and the other internally.

As the season advanced a larger number of beetles were found not carrying yeasts. Different bacteria and especially a short rod in chains were usually associated with the yeasts or obtained as the sole flora. *Aspergillus* sp., *Rhizopus* sp., and *Penicillium* sp. were also obtained from beetles, the first by far the greatest number of times.

The opinion has been frequently expressed that vinegar flies, *Drosophila* sp., are attracted to fermenting figs but do not themselves enter healthy ones and so spread the disease. The writer has observed them to enter or issue from figs which when examined afterwards were apparently healthy. Vinegar flies were caught in the same way as dried-fruit beetles and were found carrying the yeasts both externally and internally.

While the flies are attracted by the odors of fermentation and are known to feed on yeasts and decaying fruit, as reported by Baumberger (*1*), Schulze (*31*), Sturtevant (*34*), and Northrop (*22*), Baumberger has found that adult Drosophila flies oviposit on sterile fruit, the presence of yeasts being unnecessary, and Guyénot (*15*, *16*) has raised *Drosophila ampelophila* aseptically for two years (40 generations), indicating that Drosophila flies may enter sound fruit for the purpose of breeding and feeding and in so doing transmit the fig-souring organisms.

Phillips et al. (*23*) in their studies of the transmitting agents of *Aspergillus niger* causing what is called black smut in figs consider *Carpophilus hemipterus* first in importance and *Drosophila ampelophila* as second. The habitats of the dried-fruit beetle throughout the year are discussed. The writer has made observations which confirm the results obtained by Phillips. The appearance of the first sour figs in many orchards coincided with the appearance of the first beetles, and in frequent trips through the figs district of the State isolated orchards have been studied for the presence of both the beetles and souring. It seemed that wherever decaying fruit, melons, oranges, figs, apples, apricots, peaches, plums, etc., were left on the ground, offering a breeding place for the beetles and the vinegar fly, souring was invariably abundant. Young Adriatic fig orchards in sections where grain farming predominated were found free of souring and beetles It was interesting to note the souring of the first crop figs of Adriatic, Brunswick, Mission, Kadota, and White San Pedro. These come early in the season (June) when the overwintering generation of the beetle has not multiplied extensively; souring, therefore, is found only in such figs in orchards with decaying oranges, melons, or other fruits having imperfect sanitation. In connection with orchard sanitation and the presence of souring, an inquiry was made by I. J. Condit about the presence of the dried-fruit beetle in Italy where souring is reported as nonexistent. Doctor Briganti, of Portici, Italy, wrote that "*Carpophilus hemipterus* is rarely found in Italy * * * cases of fig smut and the manifestation of the fruit beetle are so sporadic and of so slight an importance as not to interest deeply our entomologists." In a populous country like Italy, fruits decaying under the trees or on the drying grounds are very scarce.

The idea has been often expressed by Eisen (*8*), Coit (*6*), Coit and Johnston (*17*), and is prevalent among growers that cold nights, overirrigation, cool damp weather, or other environmental conditions cause souring. In view of the evidence presented above, moisture variations can have no other than a modifying effect. Given the infection, carried by the dried-fruit beetle or the vinegar fly, slow drying of the fig with the accompanying slow increase in sugar concentration may favor greatly the development of the parasite. The opposite would be the case with quick drying. The yeasts would probably never develop or their activities would be quickly checked. To find out the effect of high sugar concentration on the growth of yeasts, nutrient agar was prepared to which 40 and 60 per cent of dextrose were added, instead of the customary 2 per cent. The yeasts grew readily on 40 per cent but made very poor growth on the 60 per cent dextrose agar.

SUMMARY

A destructive fermentation of figs, both caprified and parthenocarpic, is described, with a discussion of the economic importance of the disease and its geographical distribution.

Three different types of yeasts were isolated from fermenting figs; their cultural characteristics, fermentation ability, morphology, and pathogenicity are discussed.

The transmission of these yeasts into the cavities of figs by the dried-fruit beetle (*Carpophilus hemipterus*) is shown. The transmission was found to be both internal and external mechanical.

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TEST TO DETERMINE TOXICITY OF PYRETHRUM VAPORS TO HONEYBEES[1]

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INTRODUCTION

The insecticidal properties of pyrethrum flowers have been known and utilized for more than a century, but neither the chemistry of the toxic principle nor the exact method of toxication is fully understood. In experiments with pyrethrum as a possible substitute for arsenicals in the control of chewing insects it became necessary to ascertain whether or not the volatile material emanating from the flowers or extracts of pyrethrum have deleterious effect upon insects. This question arose as a result of the following experiment. Tent caterpillars (*Malacosoma americana*) transferred to apple trees sprayed 1 hour previously with an alcoholic suspension of pyrethrum flowers died within 24 hours, but when caterpillars were transferred to the same trees 5 days later they fed normally, spun their tent, passed through the third and fourth instars, and continued feeding until practically all the foliage was devoured, with no apparent injurious effects. The apple trees were growing in the greenhouse, and there was no chance for rain or wind to remove the spray material. The question therefore arose whether the cessation of toxicity was due to decomposition of pyrethrum when exposed to light or to exhaustion of the volatile material emanating from it.

REVIEW OF LITERATURE

Among the earlier investigators Hanamann (*6*) [2] concludes that the toxicity of pyrethrum is due to its ethereal oils. Dal Sie (*2*) ascribes the toxic properties to volatile acids. On the other hand, De Bellesme (*1*) states that the essential oils of pyrethrum are harmless to insects. The work of Hirschsohn (*7*) indicates that the active principle is nonvolatile, since samples of insect powder kept for several days in paper bags were as active as fresh powder. Similar results were obtained by Gillette (*4*). Eymard (*3*) distilled pyrethrum flowers with water. The distillate possessed a strong pyrethrum odor, but proved nontoxic to insects. From the results of more recent investigations carried out by Yamamoto (*19*), Staudinger and Ruzicka (*9*, *10*, *11*, *12*, *13*, *14*, *15*, *16*, *17*, *18*), and McDonnell and his associates (*8*) it seems certain that pyrethrum owes its main toxic properties to a

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[2] Reference is made by number (italic) to Literature Cited, p. 1056.

mixture of two complex esters, presumably pyrethrin I ($C_{21}H_{30}O_3$) and pyrethrin II ($C_{22}H_{30}O_5$). This, however, does not exclude the possibility that the essential oils of pyrethrum may also possess insecticidal properties.

METHODS

In order to settle this point definitely, the volatile material from finely ground flowers and extracts of pyrethrum was tested on honeybees. Two different methods were employed.

FIRST METHOD

A concentrated alcoholic extract of pyrethrum flowers possessing a strong odor of pyrethrum was placed in Petri dishes or in small beakers covered with screen wire and cheesecloth. A wire cage containing bees and a mixture of honey and water was placed over the Petri dish. The cage was then covered with a large beaker, the inner walls of which were coated with the pyrethrum extract. In this manner the bees were surrounded with pyrethrum vapors. Checks were run in a similar manner, except that the pyrethrum extract was excluded.

SECOND METHOD

Respiration chambers consisting of two large glass dishes (3 inches deep and 7 inches in diameter), made air-tight by heavy rubber bands (3 inches wide), were prepared. (Fig. 1.) A similar apparatus for growing plants under controlled atmospheric conditions has already been described in detail elsewhere (*5*). Strips of filter paper were

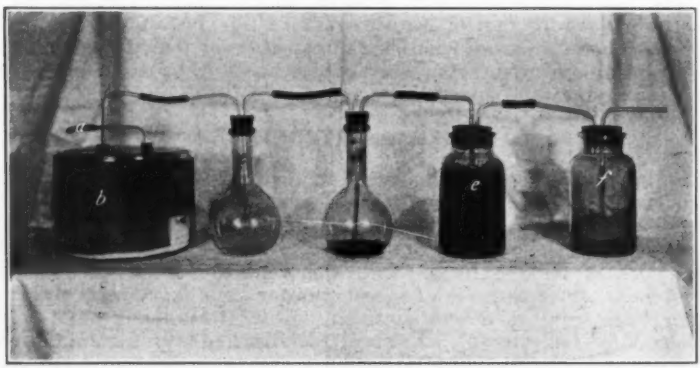

FIGURE 1.—Apparatus used in testing the effect of pyrethrum vapors upon honeybees by the second method: *a*, Tube leading to suction pump; *b*, respiration chamber; *c* and *d*, empty reservoirs to prevent entrance of dust into *b*; *e*, reagent bottle containing pyrethrum extract; *f*, reagent bottle containing ground pyrethrum flowers

placed on the bottom and sides of the dish in order to furnish convenient resting places for the bees. Inside the chamber was placed a watch glass containing cheesecloth staurated with a mixture of honey and water in equal proportions. The upper dish had two holes into which glass tubes were inserted through rubber stoppers. One glass tube was loosely plugged up with nonabsorbent cotton and connected to a series of four reagent bottles, as shown in Figure 1. Bottles *e*

and *f* contained pyrethrum extract and ground flowers, respectively, while bottles *c* and *d* were empty and served as reservoirs to prevent the dust or liquid pyrethrum from reaching the chamber. The second glass tube (*a*) from the upper dish of the chamber (*b*) was connected to a suction pump, by means of which constant aeration was maintained. The glass tubes in the reagent bottles were so arranged that the air bubbled through the pyrethrum extract. The air thus passing through the reagent bottles carried the pyrethrum vapors with it into the chamber.

From 25 to 45 bees were introduced into each chamber and allowed to remain there for 48 hours or longer. Checks were run simultaneously in identically the same arrangement, except that water was substituted for pyrethrum extract and inert powder for ground pyrethrum flowers. Extreme precautions were taken to prevent the particles of pyrethrum flowers or extract from reaching the bees in the chamber. The glass tubes were arranged in such a way that air carrying the volatile material circulated through the entire depth of the chamber before leaving it, thus assuring contact between vapor and insects.

RESULTS

Throughout the experiment the bees receiving pyrethrum vapor did not in any way differ from those in the check chambers. In each case the bees were active, fed on the honey, and appeared normal. The results presented in Table 1 indicate that the insects were not at all affected by the volatile material emanating from pyrethrum. Of the 156 bees kept in the wire cages and respiration chambers containing pyrethrum, 2 died, while of the 161 bees in the check cages and chambers 1 died, mortality being very low in each case.

TABLE 1.—*Effect of pyrethrum vapors on honeybees*

Test No.	Wire-cage method				Respiration-chamber method			
	Pyrethrum		Check		Pyrethrum		Check	
	Bees in cage	Bees dead after 48 hours	Bees in cage	Bees dead after 48 hours	Bees in chamber	Bees dead after 48 hours	Bees in chamber	Bees dead after 48 hours
1	26	0	28	0	27	0	24	0
2	25	0	32	0	41	1	44	1
3					37	1	33	0

The negative results here obtained can be explained in two ways: (1) The essential oils of pyrethrum exert no toxic effect on insects; (2) the rate of volatility may be too low to produce a lethal concentration in the atmosphere. In either case the insecticidal properties of pyrethrum vapors should be considered of no importance from the practical standpoint. If the air in the closed chamber, which was practically saturated with pyrethrum vapors (because it passed through flowers and bubbled through liquid extract of pyrethrum), did not contain a lethal dose, the possibility that the atmosphere in the open, surrounding the sprayed foliage, should contain a lethal concentration is rather remote. The cessation of toxicity of the

alcoholic pyrethrum suspension to tent caterpillars after five days' exposure on apple foliage was therefore not due to the exhaustion of the essential oils but to other factors, which possibly decomposed the active principle.

SUMMARY AND CONCLUSION

Honeybees were kept in chambers through which pyrethrum vapors were continuously circulated. At the end of 48 hours the bees appeared normal and were evidently not affected by the volatile material emanating from pyrethrum.

It appears, therefore, that the toxicity of ground flowers, as well as extracts of pyrethrum, is primarily due to the nonvolatile substances present, namely, pyrethrine I and pyrethrine II, while the essential oils do not in any way affect the insects.

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